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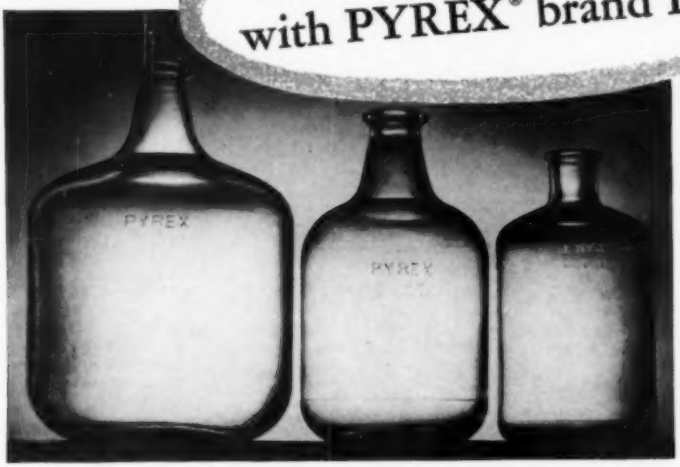

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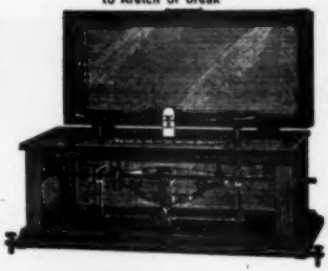

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME XLI — *JANUARY, 1952* — NUMBER 1 — CONSECUTIVE NO. 2

Terramycin Intravenous*

By W. W. ARMSTRONG, C. I. JAROWSKI, and C. J. SALIVAR†

The preparation, properties, and stability of terramycin intravenous both in the dry form and in solution are presented.

TERRAMYCIN, newest of the broad spectrum antibiotics, is a chemical substance whose chemical properties permit the preparation of a large number of acid and basic salts, organic derivatives, and complexes with organic and inorganic salts (1). Shortly after the discovery of terramycin, efforts were made to produce a dosage form of the drug suitable for intravenous administration.

From the solubility data presented in Table I, it is evident that a simple parenteral solution of high concentration is to be obtained only at high *p*H values. Thus the preparation of crystalline water-soluble alkali metal salts of terramycin would appear to offer a ready solution to the problem of preparing an intravenous dosage form. However, this approach does not utilize the superior stability and handling properties of crystalline terramycin hydrochloride. It was not until after careful study had been made of the comparative keeping properties of various dosage form mixtures containing the alkali salts and the hydrochloride, respectively, that the applicability of the latter to proper parenteral dosage formulating was recognized.

Several methods for obtaining the solubilization of terramycin at *p*H 7 were studied. Efforts were made to utilize concentrated aqueous solutions of nicotinamide, urea, and urethane. A mixture of betaine, acetamide, and triethanolamine was tried. None of these combinations could be used to dissolve adequate quantities of terramycin at neutral *p*H. Mixtures of the crystalline hydrochloride salt with various buffers were then investigated. The most favorable results were obtained with dry mixtures of amino acid sodium salts. It was essential that the buffering agent, in combination with terramycin hydrochloride, produce a solution of *p*H 9 or greater in order to effect and maintain complete solution of the antibiotic. A satisfactory preparation was resolved to be a mixture of ten parts of terramycin hydrochloride and nine parts of sodium glycinate. Upon the addition of an appropriate quantity of distilled water to this mixture, a clear solution at *p*H 9.0 to 9.25 containing 100 mg. of terramycin activity per milliliter was obtained. Extensive pharmacological animal testing indicated this buffered mixture to be entirely satisfactory as an intravenous preparation. Reports from extended clinical evaluation of this product have continued to be excellent.

Sodium glycinate from commercial sources was found to vary in purity and alkalinity. In order

* Received September 7, 1951, from the Research Laboratories, Chas. Pfizer and Co., Inc., Brooklyn, N. Y.

† The authors are grateful to Mr. R. Kersey for the biological assays, to Dr. K. Murai for the solubility figures in Table I, and to Mr. M. Marmo and Mr. J. Shaw for their technical assistance.

TABLE I.—SOLUBILITY OF TERRAMYCIN IN DISTILLED WATER AT VARIOUS *p*H LEVELS

*p*H	Potency per Milliliter, μg.
1.25	31,400[a]
2	4,600
3	1,400
4	850
5.05	525
6	700
7	1,050
8	28,250
9	38,600

[a] 1 Mg. of terramycin is equivalent to 1,000 μg. activity.

to obtain consistently the desired *p*H range of the buffered terramycin solution, it was found necessary to provide sodium glycinate of maximum purity and consistent *p*H. Parenteral grade sodium glycinate was conveniently prepared by adjustment of an aqueous solution of U. S. P. glycine to *p*H 11 with sodium hydroxide, and freeze-drying the resultant sterile alkaline solution.

TABLE II.—STABILITY OF A DRY MIXTURE OF 10 PARTS OF CRYSTALLINE TERRAMYCIN HYDROCHLORIDE WITH 9 PARTS OF SODIUM GLYCINATE

Storage Time	Temperature, °C.	Original Potency (Bioassay), %
1 hr.	100	100
18 hr.	100	96
100 hr.	100	90
1 hr.	50	100
18 hr.	50	100
100 hr.	50	100
11 mo.	50	93
11 mo.	37	96
11 mo.	Room	100

The excellent stability of a dry mixture of sodium glycinate and crystalline terramycin hydrochloride is evident from the figures in Table II and from the data in Table III. It is to be noted that prepared intravenous solutions are slow to lose potency under ordinary temperature conditions. From the latter information it is evident, therefore, that a minimal time allowance for preparation and administration of the dosage is not necessary. Progressive darkening of prepared aqueous solutions will be observed, however, during prolonged holding periods. This result of slow oxidative change, on the other hand, is not a direct indication of potency loss. A change in solution color from yellow-amber to mahogany-brown may be observed during storage under refrigeration, and as indicated in Table III, 92 per cent of terramycin activity remains after seventy-two hours.

TABLE III.—STABILITY OF AN AQUEOUS SOLUTION CONTAINING 10 PARTS OF TERRAMYCIN AND 9 PARTS OF SODIUM GLYCINATE AT A CONCENTRATION OF 100 MG. OF TERRAMYCIN PER ML.

Storage Time, Hr.	Temperature, °C.	Original Potency (Bioassay), %
2	Room	98
4	Room	96
6	Room	94
8	Room	94
24	Room	92
24	5	97
48	5	93
72	5	92

In addition to water for injection U. S. P. XIV, acceptable diluents for preparation and administration of this terramycin dosage form are normal saline U. S. P. XIV and dextrose injection U. S. P. XIV.

EXPERIMENTAL

Preparation of Parenteral Grade Terramycin Hydrochloride.—A solution of terramycin hydrochloride in pyrogen-free distilled water is sterilized by filtration. Under asceptic conditions, the filtrate is titrated with a sterile caustic solution to *p*H 5 and the slightly soluble base is separated by filtration and washed with pyrogen-free, sterile, distilled water. The amphoteric terramycin base suspended in sterile methanol is dissolved by the gradual addition of concentrated hydrochloric acid. Crystallization of sterile terramycin hydrochloride is brought about by the addition of excess acid. The sterile crystalline salt is separated, washed with sterile methanol and dried *in vacuo*.

Preparation of Parenteral Grade Sodium Glycinate.—One part of U. S. P. glycine is dissolved in three parts of pyrogen-free water and sufficient sodium hydroxide solution added to bring the solution alkalinity to *p*H 11 by the alkali "blue" electrode. The solution is sterile-filtered. The sterile filtrate is freeze-dried to a moisture content of less than 1% and milled. The sterile sodium glycinate is then ready for use.

Preparation of Terramycin Intravenous.—Under asceptic conditions, ten parts of parenteral grade terramycin hydrochloride and nine parts of parenteral grade sodium glycinate are combined and thoroughly blended. This ratio of components in 20% aqueous solution has a *p*H of 9.0 to 9.25.

REFERENCE

(1) Finlay, A. C., Hobby, G. L., P'an, S. Y., Regna, P. P., Routien, J. B., Seeley, D. B., Shull, G. M., Sobin, B. A., Solomons, I. A., Vinson, J. W., and Kane, J. H., *Science*, **3**, 85(1950).

Assay of Aminophylline-Vinbarbital Solutions*

By GEORGE F. HOFFNAGLE† and MARY L. MILLIGAN

An assay for aminophylline-vinbarbital solutions through their silver salts has been developed. This method combines the U. S. P. XIV assay for aminophylline with a modification of the Budde assay for barbiturate. The ability of the Budde assay to eliminate interference by barbiturate decomposition products is investigated and confirmed. Discussion and quantitative evaluation of sources of error in the assay are presented. One of these errors is directly applicable to the U. S. P. XIV assay of aminophylline.

THE PROBLEM of assaying barbiturate solutions for active barbiturate, i. e., unhydrolyzed barbiturate, has been approached by many workers with several different methods. The U. S. P. XIV method, that of extraction with an immiscible solvent, evaporating the solvent, and weighing the residue as barbiturate, only partially solves the problem of decomposition. Rotondaro (1) and van Itallie and Steenhauer (2) were able to separate the barbiturate from its decomposition products by a complicated and tedious series of extractions. Bell (3) discussed several reported complexes as being unreliable, the unreliability being due probably to insufficient knowledge of these complexes.

The direct alkaline titration of barbiturates as described by von Babitsch (4) does not eliminate the acidic decomposition compounds (usually stronger acids than the barbiturate), and conductimetric titration [Bartilucci and Discher (5)] would also find quantitative differentiation between the barbiturate and its decomposition products difficult.

The formation of insoluble mercury barbiturate and assay by weighing this salt [von Babitsch (4)] is subject to the errors caused by acidic decomposition.

Budde (6) developed a method of titrating barbiturates in sodium carbonate solution with silver nitrate, the end point being the first permanent appearance of a precipitate of silver carbonate. This method has been found to be quantitative for phenobarbital in the presence of the usual decomposition products (7). The method depends on the fact that silver barbiturate is soluble in sodium carbonate solution but is undissociated, whereas the silver compounds of the decomposition products are dissociated and therefore cannot hold the silver in solution. The barbiturate actually can dissolve silver carbonate under these conditions and therefore obviously is dissociated less than is the silver carbonate in the titration medium. In the case of vinbarbital,[1] the silver barbiturate fluoresces in alkaline medium and obscures the end point under the conditions of Budde's assay as modified by Jatul (7).

The cobalt colorimetric method for barbiturates reported first by Zwikker (8), modified by Koppanyi (9, 10), and made quantitative by Cohen (11), involves the development of a blue color, using as reagents cobaltous chloride and isopropylamine in anhydrous chloroform. No conclusive investigation regarding possible interference by hydrolysis products of the barbiturate has been presented.

PROCEDURE

The silver salts of barbiturates are soluble in ammonium hydroxide solution as well as in sodium carbonate solution. Therefore, the U. S. P. XIV assay for aminophylline, involving precipitation of silver theophylline and subsequent titration of the filtrate with ammonium thiocyanate for excess silver, need only be modified by the condition that sufficient silver nitrate be added to accommodate both the barbiturate and the theophylline, plus a suitable excess. This excess is required to prevent competition for the silver between the theophylline and the barbiturate.

An aliquot of the filtrate can be titrated for the theophylline assay. Another aliquot is made slightly acidic with nitric acid and is extracted with chloroform in five 25-ml. portions. The chloroform contains the barbiturate and its decomposition products. The combined chloroform extracts are evaporated to dryness in an Erlenmeyer flask on a steam bath, the residue is taken up in 5% sodium carbonate solution, a slight excess of silver nitrate added, and the solution is brought to a definite volume. Then it is centrifuged in the presence of nitrobenzene to remove the slight amount of precipitated silver carbonate, and an aliquot is made acidic and titrated with ammonium thiocyanate. The amount of silver found in the sample is converted by calculation to its equivalent of the unhydrolyzed barbiturate.

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[1] "Delvinal" is Sharp & Dohme's trade mark for this compound.

EXAMPLE OF PROCEDURE AND RESULTS

The vinbarbital acid and theophylline, U. S. P., were dried at 105° for four hours. Four mixtures of the two, representing approximately 275 mg. of vinbarbital and 1 Gm. of anhydrous theophylline in each sample, were placed in 200-ml. volumetric flasks. Each sample was treated as follows:

There were added 0.5 ml. of 95–100% ethylenediamine, 2.86 ml. of 28% reagent ammonia water, and 100 ml. of distilled water, and solution was effected. Exactly 75 ml. of 0.1 *N* silver nitrate was added, and this was followed by sufficient distilled water to make a volume of 200 ml. The mixture was allowed to stand at room temperature for one hour. (Heat, which is used in the U. S. P. XIV assay, was not employed, since this would hydrolyze some of the barbiturate.) The flask was cooled in an ice bath with occasional agitation for twenty-five minutes.

The solution was kept cold while it was filtered through a sintered filtering crucible of medium porosity. The filtrate was allowed to return to room temperature; then a 50-ml. aliquot was titrated with 0.1 *N* ammonium thiocyanate in the presence of 6 ml. of nitric acid and 2 ml. of ferric ammonium sulfate T. S. for the theophylline assay.

An aliquot (100-ml.) of the filtrate was made slightly acidic by the addition of 7 ml. of nitric acid in a separatory funnel, after which it was extracted with five 25-ml. portions of chloroform, the chloroform extracts being pooled in an Erlenmeyer flask and evaporated to dryness on a steam bath.

The residue was taken up in 40 ml. of 5% sodium carbonate solution in two portions, and the flask was washed with four 10-ml. portions of distilled water, the solutions being collected in a 100-ml. volumetric flask. There was added 1.3 ml. of 10% silver nitrate solution, followed by sufficient distilled water to make a volume of 100 ml.; then the mixture was agitated thoroughly.

The solution was placed in a centrifuge tube, 2 ml. of nitrobenzene was added, the mixture was thoroughly agitated and then was centrifuged at 2,400 r. p. m. for twenty-five minutes. An aliquot (25-ml.) was removed carefully, made acidic with 4 ml. of nitric acid, and 2 ml. of ferric ammonium sulfate T. S. was added. This sample then was titrated with 0.01 *N* ammonium thiocyanate, and the silver found was converted by calculation to vinbarbital. The results of this assay procedure may be found in Table I.

TABLE I.—ASSAY OF AMINOPHYLLINE-VINBARBITAL SOLUTIONS

Sample	Theophylline Used, Gm.	Theophylline Found, Gm.	%
1	1.1758	1.1670	99.25
2	0.9729	0.9715	99.86
3	1.0090	1.0062	99.72
4	1.0426	1.0363	99.40
Sample	**Vinbarbital Used, Gm.**	**Vinbarbital Found, Gm.**	**%**
1	0.2738	0.2711	99.01
2	0.2860	0.2837	99.20
3	0.2803	0.2843	101.43
4	0.2660	0.2653	99.74

DISCUSSION

The procedure of bringing a suspension to a definite volume and then filtering it (the initial step of this method) is subject to error due to the volume of the precipitate. By a pycnometric method, the specific gravity of a prepared sample of silver theophylline was determined to be 2.11. The error can be calculated for both determinations from the specific gravity of the silver theophylline and the assay results for the theophylline and barbiturate.

For the theophylline correction:

$$\frac{-1.6\ (\text{Gm. of theophylline found})}{\text{Sp. gr. of silver theophylline}} \times \frac{1}{2} \times \frac{\text{mg. theophylline found}}{100}$$

or

$$-0.0000038\ (\text{mg. theophylline found})^2 = \text{correction in mg. for theophylline}$$

For the vinbarbital correction, by the same reasoning:

$$-0.0000038\ (\text{mg. theophylline found})\ (\text{mg. vinbarbital found}) = \text{correction in mg. for vinbarbital}$$

These corrections are of the order of 0.4% for the amounts here assayed.

The U. S. P. XIV procedure of washing the precipitated silver theophylline could be followed if the filtrate then were brought to some definite volume. Considerably more time would be required, however, for that procedure.

It was found that the U. S. P. XIV assay for aminophylline yields low results of approximately 2.66 mg. of theophylline in the quantities here tested, due to the solubility of silver theophylline in ammonium hydroxide solution. This was determined by following the procedure with approximately 90% of the silver nitrate necessary to combine with all the theophylline used. The experiment was done in quadruplicate on the same parent solution of theophylline; two of the precipitated solutions being heated and two being allowed to stand at room temperature. The unheated samples showed titratable silver after filtration equivalent to 2.70 mg. of theophylline, while the heated samples showed titratable silver after filtration equivalent to 2.62 mg. Ammonium thiocyanate, 0.01 *N*, was used in this titration. These results cannot be considered significantly different. The point made here is that the procedure of heating the suspension of silver theophylline is not necessary from the standpoint of accuracy; it facilitates filtration only.

The solubility of silver theophylline in the solution to be filtered, using the U. S. P. XIV method of assay (i. e., addition of ammonium hydroxide), was not compared with its solubility in the absence of the ammonium hydroxide, as suggested by Griffenhagen and Brady (12). However, these values placed the error to be found in the U. S. P. XIV method on a quantitative basis.

The question of error due to the use of nitrobenzene to facilitate removal of silver carbonate from the sample for titration of silver vinbarbital was approached as follows: the solubility of the nitrobenzene in a 2% sodium carbonate solution, essentially

the solution present in the sample, was found to be 0.16% v/v. This agrees with the published solubility in water (13). To get the complete answer immediately, a parent solution of vinbarbital in 2% sodium carbonate with sufficient silver nitrate added to react with only 90% of the barbiturate was divided into four parts. The proper amount of nitrobenzene was added to two of these portions, after which they were carried through the centrifugation and titration procedures. The portions treated with nitrobenzene showed an average value of 0.14% less than the untreated portions, thus approaching the maximal dilution attributable to solubility of nitrobenzene. Since saturation would only be approached by the nitrobenzene in the procedure used, this result is quite logical and indicates that no loss of silver barbiturate is incurred due to solubility of the compound in nitrobenzene.

Corrections for the sources of error described above should be made in the following sequence:

1. +0.16% (maximum) to the vinbarbital result due to the solubility of nitrobenzene in the sodium carbonate solution.

2. +2.66 mg. to the theophylline result because of the solubility of silver theophylline in ammonium hydroxide solution.

3. A negative correction to the vinbarbital result for the volume occupied by the silver theophylline in the original volumetric solution.

4. A negative correction to the theophylline result as applied in correction No. 3.

The latter two corrections should be made using the figures corrected by No. 1 and No. 2, but should not influence each other; i. e., No. 3 should not use the value for theophylline already corrected by No. 4.

The results of these corrections on the assays tabulated above are shown in Table II.

TABLE II.—APPLICATION OF CORRECTIONS TO ASSAYS IN TABLE I

Sample	Theophylline, Found, Uncorr. %	Total Correction, %	Theophylline Found, Corr., %
1	99.25	0.21	99.04
2	99.86	0.11	99.75
3	99.72	0.12	99.60
4	99.40	0.14	99.26
Av.	99.56		99.41

Sample	Vinbarbital, Found, Uncorr. %	Total Correction, %	Vinbarbital Found, Corr., %
1	99.01	0.29	98.72
2	99.20	0.21	98.99
3	101.43	0.22	101.21
4	99.74	0.23	99.51
Av.	99.85		99.61

TABLE III.—APPLICATION OF ASSAY TO HEAT-TREATED SAMPLES

Treatment	Vinbarbital Av. 2 Samples, Mg.	% Decompn. Compared to Initial
No heat	35.88	0
1/2 hr. 120° C.	34.11	95.07
1 hr. 120° C.	32.36	90.19
2 hr. 120° C.	29.49	82.19

DECOMPOSITION OF VINBARBITAL

In order to show that this assay method will evaluate the decomposition of the barbiturate, a parent solution of vinbarbital was made using ethylenediamine as the base. This was divided into portions, two of which were not heated, the remainder being autoclaved and assayed according to the full recommended procedure. No precipitate appeared in any sample when the silver nitrate was added. The results of this experiment are shown in Table III.

These results show a positive and significant response of the assay to the hydrolysis of the barbiturate and reaffirm the validity of the silver method as described by Budde.

SUMMARY

1. An assay method for aminophylline-vinbarbital solutions is presented. This assay determines both substances through their silver salts.

2. It is shown that the method determines active, or unhydrolyzed, barbiturate. Other methods either do not or have not been shown to eliminate errors due to decomposition products of the barbiturate.

3. Sources of error in the assay are discussed and evaluated quantitatively.

4. An error inherent in the U. S. P. XIV assay of aminophylline is placed upon a quantitative basis.

REFERENCES

(1) Rotondaro, F. A., *J. Assoc. Offic. Agr. Chemists*, **23**, 777(1940).
(2) van Itallie, L., and Steenhauer, A. J., *Pharm. Weekblad*, **58**, 1062(1921).
(3) Bell, C. W., THIS JOURNAL, **30**, 240(1941).
(4) von Babitsch, S., *Pharm. Monatsh.*, **17**, 87(1936).
(5) Bartilucci, A., and Discher, C. A., THIS JOURNAL, **39**, 641(1950).
(6) Budde, H., *Apoth. Ztg.* **49**, 295(1934).
(7) Jatul, B. B., THIS JOURNAL, **33**, 217(1944).
(8) Zwikker, J. J. L., *Pharm. Weekblad*, **68**, 975(1931).
(9) Koppanyi, T., *et al.*, *Proc. Soc. Expll. Biol. Med.*, **30**, 542(1933).
(10) Koppanyi, T., *et al.*, THIS JOURNAL, **23**, 1074(1934).
(11) Cohen, E. L., *Am. J. Pharm.*, **118**, 40(1946).
(12) Griffenhagen, G. B., and Brady, E. S., THIS JOURNAL, **40**, 211(1951).
(13) "Handbook of Chemistry and Physics," 31st ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1949, p. 682.

Studies on Species of *Asclepias.* I and II. Introduction and a Comparative Study of the Roots*

By W. E. HASSAN, Jr.,† H. W. YOUNGKEN,‡ and M. W. QUIMBY§

The objectives of a pharmacognostical investigation of *Asclepias tuberosa* L., *Asclepias syriaca* L., *Asclepias speciosa* Torr., *Asclepias incarnata* L. var. *alba* Hort., *Asclepias incarnata* L. var. *pulchra* Hort., and *Asclepias curassavica* L. are discussed. Historical data and uses in medicine and pharmacy are presented. A greater part of the report deals with the comparative histologies of the roots of the above-mentioned members of the genus.

THE ROOTS of *Asclepias tuberosa* L. and allied species have been used in medicine for over a century. They have been employed as diaphoretics, expectorants, diuretics, and in the treatment of pleurisy. Throughout this period, various investigators have published gross descriptions of single species of root and preliminary histological studies.

The purpose of this portion of the investigation was to make available a comparative study of the histological characteristics of the following species of *Asclepias: A. tuberosa* L., *A. syriaca* L., *A. speciosa* Torr., *A. curassavica* L., *A. incarnata* L. var. *alba* Hort., and *A. incarnata* L. var. *pulchra* Hort.

HISTORY

The United States Pharmacopeia, in various revisions, has recognized the roots of three species of *Asclepias*. The roots of *A. tuberosa* were recognized from 1820 to 1905; those of *A. syriaca* and *A. incarnata* from 1820 to 1864 and again from 1873 to 1882. In 1916 the root of *A. tuberosa* was admitted to the National Formulary, where it remained until 1936.

A survey of the pharmacognosy literature showed that investigations upon the majority of the members of the genus have been meager.

Wall briefly described the physical characteristics of the root of *A. tuberosa* (1) and the rhizome and rootlets of *A. incarnata* (2).

Hart (3) described the histological characteristics of the root of *A. tuberosa* and two of its substitutes.

Youngken (4) described and figured the plant of *A. tuberosa* and gave the physical characteristics and histology of the dried root.

Materials and Methods.—The living plant material for this investigation consisted of the entire plants of *A. tuberosa*, *A. syriaca*, *A. speciosa*, *A. curassavica*, *A. incarnata* var. *alba*, and *A. incarnata* var. *pulchra*.

The seeds of all varieties, except *A. syriaca*, were obtained from a commercial seed house. The plants of *A. syriaca* were found growing in the wild state.

The seeds were planted in flats in ordinary topsoil, were in the open air at all times, and at eight weeks were transplanted into the open field. While in the flats they were watered with cow manure water. All the plants except *A. syriaca* were grown in this manner, in the open air, in Boston.

Specimens of the roots, stems, leaves, flowers, pods, and seeds were gathered at various times during their growth, and were examined macroscopically and microscopically, and the surplus materials were deposited in the herbarium of the Massachusetts College of Pharmacy.

Whenever possible, sections of the fresh plant organ were cut with a sliding microtome. The sections were then stained using the safranin-fast green method. Leaf, stem, and root material which could not be sectioned in the fresh state were dehydrated by the use of the tertiary butyl alcohol series, imbedded in paraffin-rubber mixture, and sectioned with a rotary microtome. The sections were then "fixed" to the slide and stained, using the safranin-fast green method.

COMPARATIVE ANATOMY OF THE ROOTS

Asclepias tuberosa.—Transverse sections of the root exhibit an outer cork layer of tangentially elongated, slightly lignified cells.

The secondary cortex consists of a broad zone of parenchyma cells, some of which contain starch grains, and others, druses of calcium oxalate. In the outer region of this zone are closely arranged rows of stone cells possessing thick, lignified walls and branching pit canals. These stone cells occur in rows of five to six, the isolated patches being rectangular in shape.

The phloem region is composed of numerous thin-walled phloem parenchyma cells. The phloem cells contain numerous druses of calcium oxalate and clusters of starch grains. The phloem rays are not very pronounced and average two cells in width. The phloem ray cells, phloem parenchyma cells, and phloem sieve cells appear to be angular in radial view and elongated in longitudinal view. Fibers are not present in the phloem.

The xylem consists of a broad zone of starch- and crystal-bearing wood parenchyma and imperforate

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tracheary elements. The xylem rays are from one to five cells in width and are composed of thin-walled, starch-bearing cells. The imperforate tracheary elements are short and sinuate with long tapering ends. The pits are few, circular to slightly oblique, and with greatly reduced borders. The vessel elements are not very numerous and are located in the wedges produced by the wood fibers. The vessel elements are very short and narrow with simple perforations in the end walls. The pits are numerous, flattened, and slit-like, giving the walls a reticulate appearance.

In transverse sections of a two-year-old root, the outermost half of the xylem exhibits wedge-shaped areas of thin-walled, lignified wood fibers. These "wedges" are separated by the xylem rays and tangential bands of wood parenchyma. The vessel elements of this outer area are arranged either singly or in groups, and are concentrated within the wedge areas of the fibers.

The lignified elements in the inner half of the xylem are rather scanty and consist of scattered groups of vessels and a few fibers. The pits on these imperforate tracheary elements are numerous, and possess reduced borders.

Scrapings of the dried root mounted in water exhibit starch grains which are simple and 2- to many-compound. The individual grains are spheroidal, plano-convex to polyhedral in shape, and possess a central hilum and indistinct lamellae. The smaller grains average 8 μ in diameter and the larger, 15 μ.

Asclepias syriaca.—Transverse sections of the root of this plant exhibit the same general histological characteristics as the root of *Asclepias tuberosa*. The following discussion focuses upon the differences observed in transverse and longitudinal sections.

An examination of transverse sections of the young root reveals that the secondary cortex is first formed in the inner region of the periderm, immediately outside the secondary phloem, as isolated cells or in very small groups. As the root matures, the secondary cortex occurs in the outer region of the periderm in scattered and irregular groups, and not in regular rows as were observed in *Asclepias tuberosa* L.

Transverse sections of very old roots exhibited tangentially elongated groups of thick-walled stone cells scattered in the outer region of the periderm and in the phloem.

The imperforate tracheary elements of the young root are thin-walled and slightly lignified, and occur in tangential bands as opposed to the radial bands observed in *Asclepias tuberosa*.

The vessel members are extremely large and thick-walled, and are more numerous in occurrence. They occur in large groups which are very uniformly distributed in the root. The end walls are more nearly transverse and possess simple perforations. The vessels exhibit typically elliptical pits with distinct borders and slit-like apertures. In some, however, the border is greatly reduced and the pit aperture is greatly elongated, so as to produce a reticulate appearance.

The roots of this plant possess latex cells and latex.

Scrapings of the dried root mounted in water exhibit starch grains which are 2- to 3-compound. The individual grains are spheroidal, plano-convex to polyhedral in shape, and possess a central hilum and indistinct lamellae. The smaller grains average 25 μ in diameter and the larger, 35 μ.

Asclepias speciosa.—Transverse sections of the root of this plant are very small, approximately 10–15 mm. in diameter.

Microscopic examination reveals that the plant possesses many latex cells, which are situated in the inner region of the cortex and in the phloem. These latex cells are very thick-walled and turn brown in 5% potassium hydroxide solution and yellow in diluted iodine solution.

Druses and starch are very prominent in the cortex, in the phloem ray cells, and in the wood rays.

Phloem fibers and stone cells are completely absent.

The vessels are rather few; they are characterized by their arrangement in a radial row across the diameter of the root.

The vessel members are very short and possess simple perforations in the end walls. The pits are slit-like, elongated, and crowded, so as to give the wall a reticulated appearance. The pit borders vary from distinct to indistinct.

The secondary xylem region is composed of xylem ray cells, thin-walled fibers, vessels, and abundant wood parenchyma. The secondary xylem is characterized by numerous rays up to seven cells in width. All the ray cells contain starch, and a few of them contain druses. The fibers and the wood parenchyma are arranged in radially alternating groups. The fibers are very short and possess numerous large, circular to elliptical pits with distinct borders.

Scrapings of the dried root mounted in water exhibit starch grains which are single in occurrence. The individual grains are spheroidal, plano-convex to polyhedral in shape, and possess a central hilum and indistinct lamellae. The smaller grains are approximately 2 to 4 μ in diameter and the larger grains, 20 μ.

Asclepias incarnata var. pulchra.—Prior to a discussion of the anatomy of this root, it is of prime importance to note that this cultivated plant does not develop a persistent primary root. The entire root system is composed of a dense growth of adventitious roots.

An interesting feature of this root is that many root hairs are to be observed for some distance back of the calyptra.

Transverse sections of the root exhibit a small quantity of periderm formation. The periderm cells are thick-walled, tangentially elongated, and slightly lignified.

Occasionally one will observe cells in the hypodermis which are distinctly different from the surrounding hypodermal cells. They are characterized in transverse section by having their outer walls and the outer third of the radial walls thickened and lignified. The inner two-thirds of the cell is pushed inwards by the two adjacent cells.

In surface view, these cells appear to be rectangular in shape and unequally thickened. They differ from stone cells by the absence of pit canals.

Transverse sections exhibit a triarch arrangement of the vascular cylinder (Fig. 1).

The remaining tissues and elements are typical of dicotyledonous root structure and are therefore not described.

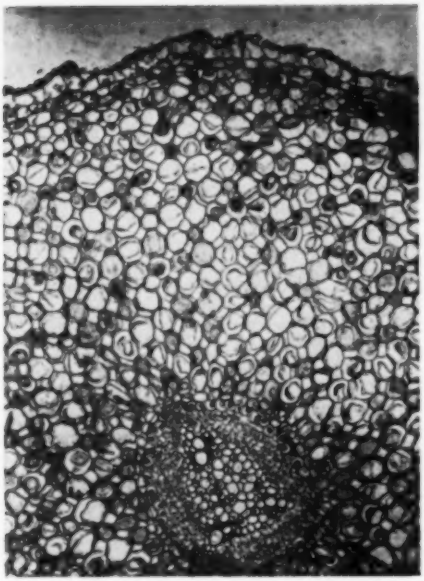

Fig. 1.—Transverse section of the root of *Asclepias incarnata* var. *pulchra* showing the triarch arrangement of the vascular cylinder.

The starch grains are simple and 2- to 5-compound. The individual grains are spheroidal to polyhedral in shape, and possess a distinct central hilum and indistinct lamellae. The grains in this variety are exceptionally small. The majority observed measured between 2 and 4 μ in diameter.

Asclepias incarnata var. alba.—The root development of this plant is very similar to that of *Asclepias incarnata* var. *pulchra* in that the entire root system is composed of adventitious roots.

The epidermis consists of a single layer of thin-walled, tangentially elongated cells possessing root hairs.

The hypodermis consists of large radially elongated cells.

This root still maintains the primary cortex, a condition quite unusual in roots exhibiting secondary dicotyledonous structure.

A thick-walled endodermis is present because of the lack of periderm formation.

The primary vascular tissue possesses either a triarch or tetrarch type of arrangement. The cambial cells arising in the radii of the primary phloem patches give rise on their inner faces to cells which develop into rather wide wedge-shaped xylem rays. This results in the formation of a 3- to 4-armed cross arrangement of the wood tissue.

The amount of secondary development of the vacular tissue is quite scanty.

The starch grains are simple and 2- to 3-compound. The individual grains are spheroidal to polyhedral in shape, and possess a distinct central hilum and indistinct lamellae. The majority of the starch grains observed measured between 4 and 8 μ in diameter.

The remaining tissues and elements are similar to those which are described under the other members of the genus.

Asclepias curassavica.—The root development of this plant is similar to those of *A. incarnata* var. *alba* and *A. incarnata* var. *pulchra* in that the entire root system, of the plants used in this study, is composed of adventitious roots.

The outer layers are composed of moderately thick-walled slightly lignified cells. The periderm is of epidermal origin in this root.

A primary cortex is present, a condition very unusual in roots exhibiting secondary dicotyledonous structure. Many of the cells of the primary cortex contain large druses, and others contain numerous spheroidal starch grains.

A moderately thick-walled endodermis is present.

The vessel members are very short and possess simple perforations in their end walls. The pits are slit-like, elongated, and crowded so as to give the wall a reticulated appearance. The pit borders vary from distinct to indistinct.

The secondary xylem region is composed of xylem ray cells, thin-walled fibers, vessels, and wood parenchyma. The secondary xylem is characterized by numerous rays, which are up to five cells in width.

The imperforate tracheary elements are short and moderately sinuate with long tapering ends. The pits are few, circular to slightly oblique, with greatly reduced borders.

The starch grains of this species were observed to occur singly. The grains were spheroidal to polyhedral in shape, and possessed a distinct central hilum and indistinct lamellae. The majority of the grains observed measured from 4 to 15 μ.

SUMMARY

1. Histological studies have been conducted upon the roots of six species of *Asclepias*.

2. As a result of the comparative histology, each of the species described can be microscopically distinguished from the medicinally employed *A. tuberosa* and from each other.

3. The histology of the roots of *Asclepias incarnata* var. *alba* and *Asclepias incarnata* var. *pulchra* is presented for the first time.

REFERENCES

(1) Wall, O. A., "Handbook of Pharmacognosy," 4th ed., C. V. Mosby Co., St. Louis, Mo., 1917, p. 187.
(2) Wall, O. A., *ibid.*, p. 232.
(3) Hart, F., *C. U. C. P. Alumni J.*, **23**, 154(1916).
(4) Youngken, H. W., "A Textbook of Pharmacognosy," 6th ed., The Blakiston Co., Philadelphia, Pa., 1948, p. 686.

Studies on Species of *Asclepias*. III. A Comparative Study of the Stems*

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The comparative anatomy of the stems of *Asclepias tuberosa* L., *Asclepias syriaca* L., *Asclepias speciosa* Torr., *Asclepias incarnata* L. var. *alba* Hort., *Asclepias incarnata* L. var. *pulchra* Hort., and *Asclepias curassavica* L. is presented. Only minor differences occur in the histologies of the stems of the species of *Asclepias* examined. Similarities were noted in the absence or scantiness of periderm, the presence of druses and abundant starch, a pericycle in the form of isolated groups of nonlignified or slightly lignified fibers, a siphonostelic arrangement of vascular tissue, an endodermis observable only in iodine mounts, and internal phloem patches. Differences were noted in the number of latex vessels, and in the distribution of vessels in the secondary xylem.

THIS PAPER, the third in a series on the genus *Asclepias*, deals with the comparative anatomy of the stems of six species or varieties, namely; *A. tuberosa* L., *A. syriaca* L., *A. speciosa* Torr., *A. incarnata* L. var. *pulchra* Hort., *A. incarnata* L. var. *alba* Hort., and *A. curassavica* L.

In the microscopical part of this investigation, both temporary and permanent mounts were used. These mounts were prepared by the usual techniques.

There is a marked anatomical similarity in the stems of the species named. For this reason, we shall emphasize common generic characteristics, and shall point out differences among species where they occur.

COMPARATIVE ANATOMY

Although *Asclepias* is a perennial plant, a periderm is lacking except as a covering over the transitional region between stem and root. Even here it is poorly developed. The major portion of the stem is covered by an epidermis, which is nondistinctive except for a thin cuticle. Hairs, when present, are morphologically similar to those found on the leaves, and are described in a paper dealing with that organ.

A distinct hypodermis is discernible only in the stems of *A. incarnata* vars. *pulchra* and *alba*. Here the cells are slightly larger than those of the adjacent cortex.

The primary cortex is narrow, and is composed of small, thin-walled, typically parenchymatous cells, some of which contain druses, others starch. In addition, latex vessels are observable in all species except *A. tuberosa*. They are most numerous in *A. speciosa*.

In our anatomical studies of the stems of this genus, the most difficulty, as far as interpretation is concerned, was encountered in the endodermis (Fig. 1). This tissue is defined as the innermost layer of the cortex, and is so designated if its cells are morphologically distinct from those of the rest of the cortex. Permanent, stained slides do not reveal distinctly the presence of such a layer. However, sections stained with a weak iodine solution show that those cells comprising the innermost layer of the cortex, or what corresponds anatomically to an endodermis, are packed with starch. The blue color imparted to the contents of these cells sets this layer off as being most distinctive. The presence of abundant starch in the endodermal cells of stems and roots occurs rather commonly in other plants. For this reason, we have suggested the presence of an endodermal region in the stems of *Asclepias*, and believe it to be a contributing factor in its identification. An interrupted ring of pericyclic fibers, nonlignified except in the older stems, is located just within the endodermis.

The vascular tissue displays a siphonostelic arrangement, despite the herbaceous habit of *Asclepias* stems.

Numerous isolated patches of primary phloem are present on the periphery of the narrow secondary phloem. The secondary phloem itself is nondistinctive except for being continuous, and for containing uniseriate phloem rays, many of which contain druses.

The bulk of the secondary xylem is composed of wood fibers which have thin walls. Wood parenchyma is very scanty and scattered. The vessels are not numerous, and usually arranged in radially disposed clusters of two to five, rarely up to eight. Some value for species differentiation may be placed in their distribution. In *A. syriaca* and in *A. speciosa*, for example, the vessels are concentrated in four groups, approximately equidistant apart. In *A. tuberosa*, the vessels in the peripheral area of the xylem are much larger than those in the inner region. In *A. incarnata* vars. *alba* and *pulchra*, they are uniform in distribution and in size, while in *A. curassavica*, they tend to be more numerous and to

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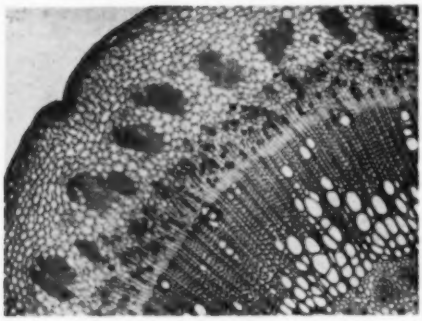

Fig. 1.—Transverse section of the stem of *A. syriaca*. × 30.

occur in longer radial clusters. The vessel members of all species, when observed in longitudinal view, are very similar. Each possesses a single perforation in the end wall and numerous small, crowded bordered pits on the side walls.

The xylem rays are predominantly uniseriate, rarely biseriate.

In the pith region, which is large in all species, numerous small patches of internal phloem may be observed forming an interrupted peripheral ring. The remaining portion of the pith is made up of typical parenchymatous cells, many of which contain druses and starch. Latex vessels are present in all species except *A. tuberosa*.

SUMMARY

A report is given of the results of a morphological study of the stems of *Asclepias tuberosa*, *A. syriaca*, *A. speciosa*, *A. incarnata* var. *pulchra*, *A. incarnata* var. *alba*, and *A. curassavica*.

The following morphological similarities may be recounted as being characteristic for the stems of those species of *Asclepias* studied:

(*a*) Absence of periderm.

(*b*) The presence of druses in the cortex, phloem rays, and pith.

(*c*) The presence of an interrupted ring of pericyclic fibers, nonlignified except in older stems.

(*d*) The presence of a starch-bearing endodermis observable in iodine-stained mounts.

(*e*) A siphonostelic arrangement of the vascular tissue.

(*f*) Vascular rays predominantly uniseriate.

(*g*) Internal phloem.

The following morphological differences may be recounted in those species of *Asclepias* studied:

(*a*) *A. tuberosa* stems are lacking in latex.

(*b*) A distinct hypodermis is discernible only in the stems of *A. incarnata* vars. *pulchra* and *alba*.

(*c*) The vessel distribution varies with each species.

Solubilizing Action of Caffeine on Benzoic Acid*

By T. HIGUCHI and D. A. ZUCK†

As a part of a study of the behavior of pharmaceuticals in solution, the reaction between benzoic acid and caffeine to form a complex was investigated. Experimental data were obtained supporting the existence of the complex in solution and the equilibrium constant for the reaction was determined.

ALTHOUGH the chemical composition of the ingredients entering into various pharmaceutical preparations is fairly well known, only limited attention has been given to possible interactions among the components, except in cases accompanied by gross chemical or physical manifestations. Rather stable complex formation may take place between some of the ingredients, but if no precipitation occurs, the existence of such complexes is usually not known or is ignored. Examples of the more well-known types of interactions are citrate ion with calcium ion, theophylline with certain barbiturates, picric acid with certain hydrocarbons, etc. Such weak complex formations often perform useful pharmaceutical purposes by solubilizing drugs or suppressing unwanted side reactions by depressing the thermodynamic activity of a drug component.

The present study covers the interaction between caffeine and benzoic acid. No attempt has been made to find an application or direct use for

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this complex formation, but a study of the nature and magnitude of the equilibrium involved has been made. It is hoped that this work will provide an insight into the character of the intermolecular forces and the relative stability of complexes of this type.

PAST WORK

Although it is generally recognized that caffeine forms complexes with a large number of pharmaceuticals, only minor studies have been carried out on the equilibria involved in the formation of the complexes. The most comprehensive investigation in this area is that of Emery and Wright (1) in 1921, covering the solubility and distribution coefficient, of caffeine in the presence of a number of complexing agents including sodium benzoate, sodium salicylate, citric acid, etc. No attempt was made by these workers, however, to analyze mathematically the equilibria involved. No mention was made of interaction of caffeine with benzoic acid. In 1937 Chambon, *et al.* (2), reported a phase rule study of the caffeine-sodium benzoate-water system. He made no attempt to obtain quantitative data relative to the stability of the complexes postulated.

THEORY

The nature and stability of the complexing reaction can be defined at any given temperature if the equilibrium constant of the reaction can be experimentally obtained. The reaction, according to the present work, takes the following form:

$$\text{caffeine} + \text{benzoic acid} \rightleftharpoons \text{caffeine} - \text{benzoic acid} \quad (1)$$

The equilibrium constant for the reaction is then

$$K_c = \frac{[\text{caffeine} - \text{benzoic acid}]}{[\text{caffeine}][\text{benzoic acid}]} \quad (2)$$

The constant can be evaluated if all the concentrations appearing in Eq. 2 can be determined directly or indirectly for an aqueous solution of caffeine and benzoic acid.

The free benzoic acid content can be obtained by having the aqueous phase in equilibrium with an immiscible nonpolar solvent, e. g., Skellysolve-C. If the distribution coefficient of benzoic acid is known between this solvent and water, the free nonionized benzoic acid content of the aqueous phase can be determined by analyzing the acid content of the organic phase. The complex concentration together with small amounts of ionized acid, corresponding to benzoate ion and benzoate-caffeine complex resulting from the ionization of the respective weak acids, can then be obtained as the difference between the total acidity of the aqueous phase, a quantity which can be readily determined by direct titration, and the free benzoic acid concentration. The small ionized acid concentrations can be approximated if it is assumed that the ionization constant of the caffeine-benzoic acid complex is roughly the same as that of benzoic acid, an assumption which will be shown later to be substantially valid. The free caffeine concentration can be determined by subtracting the complex concentration from that of caffeine originally present.

Thus by evaluating the partition coefficient of benzoic acid in a caffeine-free system, and determining the acid concentration of the organic layer, it should be possible to calculate the equilibrium constant for Eq. 1. The experimental methods employed and the results obtained by application of this approach are covered in the following section.

EXPERIMENTAL

Determination of Partition Coefficient of Benzoic Acid, Water-Skellysolve-C System.—Analytical grade benzoic acid was weighed into 125-ml. glass-stoppered bottles. Fifty milliliters of redistilled water and 50 ml. of redistilled Skellysolve-C were then added. Bottles were placed in a constant temperature water bath at 0° and were subjected to continuous shaking for two hours. The bottles were removed and placed in an ice bath to enable the two layers to separate. A 25-ml. aliquot portion from each layer was titrated with a standardized 0.025 *N* alcoholic KOH solution with α-naphtholbenzein as the indicator. To sharpen the end point, 10 ml. of absolute alcohol was added to the Skellysolve portion before titration. Blank titrations were made on the redistilled water and the redistilled Skellysolve-C. All titrations were carried out under nitrogen.

Determination of the Degree of Interaction Between Caffeine and Benzoic Acid.—The degree of interaction between benzoic acid and caffeine was determined in a manner similar to the above procedure. Several series of determinations were made. For the first, varying amounts of caffeine (0.05 to 0.45 Gm.) were added to 0.150 Gm. of benzoic acid and the analyses were carried out as described. The series of runs were then repeated with 0.250 Gm. and 1.00 Gm. of benzoic acid.

RESULTS AND DISCUSSION

The partition coefficient of benzoic acid at 0° determined experimentally is shown in Fig. 1 as functions of concentration of the acid and of sodium chloride. For the calculation of the partition coefficient the equilibrium reaction was assumed to be

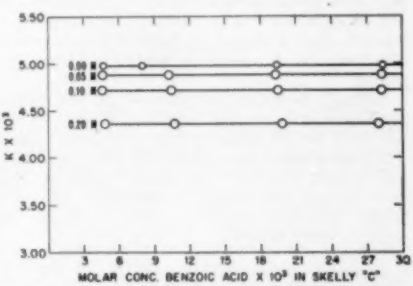

Fig. 1.—A plot of partition coefficient of benzoic acid between Skellysolve-C and water at 0° as defined by equation

$$K = \frac{(C_6H_5COOH)^2 \text{ water}}{(C_6H_5COOH) \text{ Skellysolve}}$$

Several curves refer to determination in different concentrations of sodium chloride as indicated.

TABLE I

Original Concn. of Caffeine	Total Acid Found	Total Nondissoc. Acid	Free Benzoic Acid	Complex	Equilib. Constant
Represented by Dotted Line, Fig. 2. Amount of Benzoic Acid Added Was 1.0 Gm.					
0.261×10^{-2} molar	13.69×10^{-3} molar	12.79×10^{-3} molar	11.93×10^{-3} molar	0.86×10^{-3} molar	41.2
0.519	14.64	13.71	11.96	1.76	42.6
1.030	16.23	15.25	11.92	3.33	40.1
1.541	17.92	16.89	11.94	4.94	39.6
2.060	19.56	18.48	11.94	6.54	38.9
2.691	21.53	20.40	11.94	8.46	38.4
3.090	22.85	21.69	11.93	9.76	40.6
Represented by Solid Line, Fig. 2. Amount of Benzoic Acid Added Was 0.15 Gm.					
0.516×10^{-2} molar	10.57×10^{-3} molar	9.79×10^{-3} molar	8.51×10^{-3} molar	1.28×10^{-3} molar	38.7
1.032	11.41	10.60	8.19	2.41	37.3
1.550	12.29	11.44	7.98	3.46	35.9
2.065	13.05	12.17	7.71	4.47	35.8
2.584	13.76	12.86	7.48	5.38	35.1
3.092	14.34	13.42	7.26	6.16	35.7
3.605	14.87	13.93	6.99	6.94	34.2
4.137	15.46	14.51	6.76	7.74	34.2
4.659	15.94	14.97	6.57	8.41	33.6
Represented by Broken Line, Fig. 2. Amount of Benzoic Acid Added Was 0.25 Gm.					
0.516×10^{-2} molar	14.13×10^{-3} molar	13.22×10^{-3} molar	11.62×10^{-3} molar	1.60×10^{-3} molar	38.5
1.030	15.48	14.52	11.33	3.19	39.6
1.552	16.69	15.70	11.08	4.62	38.2
2.064	17.87	16.84	10.82	6.02	38.1
2.585	18.99	17.93	10.57	7.36	39.8
3.099	19.98	18.89	10.90	8.55	34.9
3.604	20.86	19.74	10.06	9.68	36.5
4.127	21.89	20.75	9.85	10.91	36.5
4.638	22.83	21.66	9.65	12.01	36.2

$$(C_6H_5COOH)_2 \rightleftharpoons 2C_6H_5COOH \qquad (3)$$
(Skellysolve) (Water)

and the following equation

$$K = \frac{(C_6H_5COOH)^2 \text{ water}}{(C_6H_5COOH) \text{ Skellysolve}} \qquad (4)$$

was used. The benzoic acid concentration in water was corrected for dissociation.

As the curves in Fig. 1 indicate, the coefficient is substantially independent of the concentration. The salt effect, which was studied to determine the relative influence of ionized products of benzoic acid on the coefficient, appears to be fairly small below 0.05 molar.

With the partition coefficient of the acid known, the degree of interaction between caffeine and benzoic acid can be calculated on the basis of Eq. 2. The experimental data which are given in Table I yield a fairly constant value for the equilibrium constant over a wide range of component concentrations. A sample calculation of one of the constants is given below.

Experimentally determined total molar concentration of acid found in the water layer.....	$= 21.53 \times 10^{-3}$
Molar concentration of total undissociated acid in the water layer assuming K_a of 6.30×10^{-5}.....	$= 20.40 \times 10^{-3}$
Experimentally determined molar concentration of acid in Skellysolve-C	$= 28.43 \times 10^{-3}$

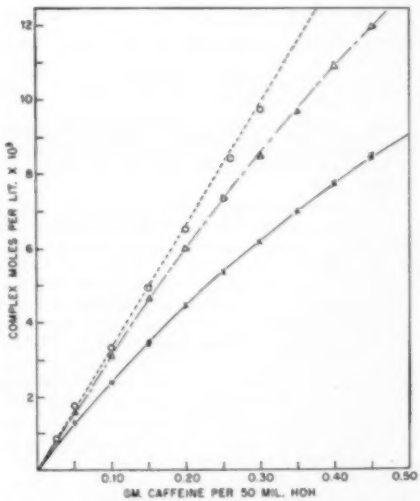

Fig. 2.—A plot of the concentration of complex formed against caffeine concentration.
○—1.0 Gm. of benzoic acid in 50 ml. of aqueous phase.
△—0.25 Gm. of benzoic acid in 50 ml. of aqueous phase.
X—0.15 Gm. of benzoic acid in 50 ml. of aqueous phase.

Partition coefficient value for a 28.43×10^{-3} molar concentration of acid in Skellysolve layer (from Fig. 1), moles/liter ..... $= 5.02 \times 10^{-2}$

Therefore by Eq. 4, molar concentration of free benzoic acid in the water layer is:

$$\sqrt{5.02 \times 10^{-2} \times 28.43 \times 10^{-3}} = 11.94 \times 10^{-3}$$

Molar concentration of complex is:

$$20.40 \times 10^{-3} - 11.94 \times 10^{-3} = 8.46 \times 10^{-3}$$

Original molar caffeine concentration ..... $= 2.691 \times 10^{-2}$

Therefore molar concentration of free caffeine is:

$$2.691 \times 10^{-2} - 8.46 \times 10^{-3}. = 1.845 \times 10^{-2}$$

Therefore "K" equilibrium constant for complex formation (Eq. 1 and 2) is:

$$\frac{8.46 \times 10^{-3}}{1.845 \times 10^{-2} \times 11.94 \times 10^{-3}} = 38.4$$

The validity of the assumption that the dissociation constant of benzoic acid and that of the complexed benzoic acid are identical is borne out by the constancy of the last column in the table. This is also supported by the fact that the stability constant calculated from the data of Emery and Wright (1) for the benzoate ion and caffeine is approximately 20 at 25°. It is therefore reasonable to assume that it will be close to that of benzoic acid at 0°. Supporting evidence has also been obtained from potentiometric studies on the effect of caffeine on mixtures of benzoate ion and benzoic acid.

In Fig. 2, the amount of caffeine-benzoic acid complex formed from varying amounts of caffeine for several concentrations of benzoic acid is given. All the observed results can be predicted with fair accuracy if the K for Eq. 2 is taken to be 37.5. Slight deviations from a constant K value may be attributable to the fact that caffeine exists to a certain degree in a polymeric form in the aqueous solution.

REFERENCES

(1) Emery, W. O., and Wright, C. D., *J. Am. Chem. Soc.*, **43**, 2323(1921).
(2) Chambon, M., Bouvier, J., and Duron, P., *J. pharm. chim.*, **26**, 216(1937).

The Metabolism of Dormison (3-Methyl-pentyne-ol-3, Methyl Parafynol) and Methods for the Estimation of Dormison in Biological Material*

By PRESTON L. PERLMAN and CAROL JOHNSON

Methods are described for the estimation of Dormison in biological fluids and tissues. A major step in the metabolism of Dormison is the destruction of the ethinyl group which occurs very rapidly in the dog as revealed by the rapid decline in blood level, low level of elimination in the urine, and the *in vitro* demonstration, in rat tissues, of the disappearance of this structural group in the presence of tissue slices. No evidence of storage or accumulation of the drug in the tissues was found.

THE PURPOSE of this investigation was to study the metabolic fate of the short-acting hypnotic, Dormison[1] (3-methyl-pentyne-ol-3, methyl parafynol). This compound, a tertiary carbinol, is of particular pharmacological interest because of the ethinyl group present in the molecule. The fate of the triple bond in the body metabolism appears to be unknown.

A preliminary statement (1) on Dormison metabolism has already appeared. The present contribution deals with the detailed aspects of the study.

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[1] Registered trade mark of Schering Corporation, Bloomfield, N. J.

METHODS

The estimation of Dormison depends upon the formation of an insoluble silver derivative, elimination of excess silver ions, and liberation and quantitative colorimetric determination of the silver originally present in the complex. The formation of the silver derivative, under the conditions selected, is specific for acetylenic compounds. Since two moles of silver react with each acetylenic group (2), the concentration or amount of drug in a sample can be readily determined.

Reagents

0.10 N $AgNO_3$.—This solution should be freshly prepared each week and stored in a dark brown bottle.

6.0 N NaOH.

Alkaline Silver Reagent.—To a 250-ml. volumetric flask, add 125 ml. of 0.10 N $AgNO_3$ solution and

then 15 ml. of 6 *N* NaOH solution. Sufficient concd. NH_4OH is added to dissolve the precipitate and then 10.0 ml. of concd. NH_4OH is added in excess and the solution adjusted to volume with distilled water. Store in a dark brown bottle and prepare fresh each week.

Silver Standard.—Prepare a stock solution with a silver concentration of 0.2 mg./ml. by dissolving 157.45 mg. of $AgNO_3$ in 500 ml. of distilled water in a volumetric flask. This solution should be stored in a dark brown bottle and should be made up each week.

A working standard is prepared by making a 1:10 dilution of the stock solution with distilled water (20 μg. Ag/ml.).

Rhodanine Solution.—Prepare a saturated solution of *p*-dimethylaminobenzalrhodanine (Eastman Kodak) in acetone and filter it. A 1:5 dilution of the saturated solution is prepared with acetone for use as a diluent in the colorimetric determination of silver.

Ammonium Acetate-Gelatin Solution.—To 100 ml. of a 1.0% filtered aqueous gelatin solution, add 25 Gm. of ammonium acetate, and stir until the salt is dissolved in the gelatin solution.

Procedure

Colorimetric Determination of Silver.—A 1–4 ml. aliquot (containing 0–80 μg. of silver) is pipetted into a 15-ml. colorimeter tube. Adjust the volume of the aliquot to 5 ml. with distilled water. At the same time prepare a distilled water blank as well as a group of standard silver solutions at several concentrations within the range of 0 to 80 μg. Ag.

Add 1.0 ml. of gelatin-ammonium acetate solution followed by 2.0 ml. of diluted rhodanine dye solution to each tube. Mix the contents of the tubes by tapping the tubes gently against the palm of the hand. Read the optical density at 550 mμ within five minutes in a colorimeter (Coleman) which has been set at zero optical density with a blank consisting of all the reagents and containing 5.0 ml. of distilled water instead of silver solution.

A linear plot of the optical density against silver concentration passes through the origin and follows the Lambert-Beer law closely within the range employed (0–80 μg.). From such a curve the concentration of the unknown solutions are calculated. Although it has been found through the course of several hundred analyses and by many repetitions of the standard curve that the optical densities are repeatable for a given concentration from day to day, it was current practice to run standards with each group of unknowns.

Urine.—Urine collected in the presence of 2 ml. of concd. HNO_3 is filtered and a 75-ml. aliquot is added to a 200-ml. glass-stoppered centrifuge bottle. Adjust the *p*H to approximately 8.0 by adding concd. NH_4OH. Add 75 ml. of fresh ethyl ether to the contents of the bottle, seal the stopper with cellophane tape, and vigorously agitate the contents on a mechanical shaker for fifteen minutes. Carefully transfer the entire contents of the bottle to a separatory funnel and run the lower (aqueous) layer, along with any emulsion present, back into the centrifuge bottle. Transfer the upper (ether) phase to a second, clean glass-stoppered centrifuge bottle. Repeat the extraction of the urine and emulsion layers with an additional 75 ml. of fresh ether, but this time keep the emulsion separate. The emulsion should be transferred to a small tube and centrifuged. Collect all the ether phases in the second centrifuge bottle.

To form the silver complex, add 4 ml. of the alkaline silver reagent (sufficient for 8.7 mg. of drug)[1] to the combined ether extracts, stopper and seal the bottle, and mix the contents vigorously on a mechanical shaker for thirty minutes. If a large amount of precipitate is formed, add additional alkaline silver reagent. When shaking is completed, the contents are centrifuged to pack the complex firmly. Pour off the ether layer and remove the remaining traces of ether by blowing a stream of air over the surface of the aqueous phase. The silver complex will be seen floating on the surface of the aqueous layer. Suspend the complex in 15 ml. of distilled water and recentrifuge. Pour the supernatant layer through filter paper retaining as much complex as possible in the bottle. Resuspend and centrifuge the contents of the bottle twice more with water. After the washing process is completed, transfer any precipitate which may have collected on the filter paper to the main mass of complex in the bottle using small amounts of distilled water. Add 2.0 ml. of concd. HNO_3 to the silver complex, immerse the bottle in a steam bath for one hour to decompose the complex, and then filter the contents into a 250-ml. glass-stoppered volumetric flask, rinsing the bottle several times with distilled water. Adjust the *p*H of the flask contents to 7.0 (Hydrion paper) using concd. NH_4OH and then adjust the contents to volume with distilled water.

Recovery experiments using the above procedure revealed that 100–101% of the added drug could be recovered when 4.0 to 8.0 mg. was added to 75 ml. of filtered dog or human urine. Experiments also revealed that the presence of HNO_3 in the urine in no way affected the recovery of the drug. However, due to the volatility of the drug it is necessary that extreme precautions be taken to avoid loss of the compound by evaporation from the collecting vessel.

Blood.—A measured volume (5–10 ml.) of blood is transferred to an oxalated, graduated 15-ml. centrifuge tube. After it is well oxalated, the blood is transferred to a 125-ml. separatory funnel, the tube is rinsed three times with 10 ml. of distilled water, and the washings added to the contents of the funnel. If necessary, the *p*H is adjusted to approximately 8.0 with concd. NH_4OH. The contents of the funnel are then extracted three times with 50 ml. portions of fresh ethyl ether and the ether extracts collected in a 200-ml. glass-stoppered centrifuge bottle. The procedure from this point is identical with that used for urine.

Recovery experiments indicated a mean recovery of 73% of the added material. All experimental data have been evaluated with this recovery value taken into consideration.

Tissues.—Following decapitation and exsanguination of the animal, the required tissues are rapidly removed to a dish containing crushed ice. Each organ is rapidly weighed and minced with four

[1] If more than 8.0 mg. of Dormison is present, it is necessary to increase the amount of silver reagent accordingly. As to the lower limit of absolute sensitivity, it was found that quantities of Dormison smaller than 1.0 mg. could not be quantitatively recovered consistently.

TABLE I.—URINARY EXCRETION OF DORMISON BY DOGS FOLLOWING ORAL ADMINISTRATION

Animal No.	Body Wt., Kg.	Dose, Mg./Kg. Body Wt.	Collection Period, Hr.	Drug Found, Mg.	Total Dose Found, %	Total Found, %
I	10.8	200	0–1	1.4	0.1	..
			1–24	14.7	0.7	0.8
II	6.9	322	0–24	11.4	0.5	0.5
III[a]	12.0	200	0–7	21.8	0.9	..
			7–14	18.0	0.8	1.7
IV	12.4	200	0–24	14.2	0.6	..
			24–72	66.3	2.7	3.3

[a] Animal given pentobarbital (25 mg./Kg.) first and then catheterized.

volumes of ice-cold 0.02 *N* NaF in a chilled Dounce homogenizer or Waring blendor, depending upon the amount of tissue used. The resulting homogenate is then transferred to a 15-ml. pointed centrifuge tube using cold 0.02 *N* NaF to rinse the homogenizer. The centrifuge tube is capped with Parafilm and the contents mixed by inverting the tube several times. The tube is kept cold and then briefly centrifuged. The supernatant layer is transferred to a 125-ml. separatory funnel and the residue washed two more times with 10-ml. amounts of 0.02 *N* NaF. The supernatant layers after each washing and centrifuging are added to the separatory funnel containing the initial extract. The *p*H of the funnel contents are adjusted to *p*H 8 using concd. NH_4OH and the contents extracted three times with 50-ml. quantities of ether and the ether collected in a 200-ml. glass-stoppered centrifuge bottle. The formation of the silver complex and estimation of the drug is then carried out as described above for urine.

Appropriate experiments showed that it was possible to account for 90–108% of added drug from brain, spleen, fat, muscle, or kidney tissue. With liver, recoveries of the order of only 70% were realized despite many modifications in procedure.

Studies with Tissue Slices.—Organs removed for preparation of slices are kept on crushed ice until sliced. Slices are prepared with a Stadie-Riggs microtome. In the case of the liver and kidney, the outermost slice is discarded. Brain cortex slices only are used. Two hundred milligrams of tissue slices is used for each flask and is transferred, after weighing on a torsion balance, to 10-ml. Erlenmeyer flasks. Four milliliters of cold, glycine-NaOH buffer (0.13 *M*) is added to the tissue slices and the flask is stored in crushed ice until ready for incubation. The tissue control flasks (containing tissue and buffer) are immediately immersed in a boiling water bath for ten minutes, evaporation was minimized by covering the mouth of the flask with a glass marble.

To the experimental flasks, add 1.0 ml. of a Dormison solution containing 4.32 mg. of the drug, the final molarity of the drug being 0.0088 *M*. The flasks are capped with Parafilm, the contents mixed, and the vessels then placed in a 37.5° water bath.

At the end of the incubation period, the flask is placed in an ice bath and the contents are then transferred to an ice-cold, 15-ml. graduated centrifuge tube. Each flask is washed out several times with ice-cold distilled water. The tubes are centrifuged in the cold, the supernatant layer poured into another cold 15-ml. centrifuge tube, and the few drops remaining on the lip of the first tube washed into the second tube. The amount of drug in the supernatant layer is then estimated by adding 4.0 ml. of alkaline silver reagent to each tube, mixing the contents well and permitting the tubes to stand five minutes before centrifuging. The tubes are centrifuged for forty-five minutes to pack the complex firmly and the supernatant liquid is poured off. The precipitate is washed with 10 ml. of distilled water; a thin glass stirring rod is employed to suspend the precipitate in the wash water. The contents are again centrifuged for forty-five minutes, the precipitate washed with 10 ml. of water, centrifuged, and the supernatant liquid is discarded.

The silver complex remaining in the tube is then decomposed by the addition of 2.0 ml. of concd. HNO_3 and the tube immersed in a boiling water bath for approximately one-half hour. The remainder of the procedure for determination of the silver is identical with that described above.

Although it was possible to obtain satisfactory recoveries (94 and 93% for brain and kidney, respectively) with liver, only 73% of the Dormison could be recovered even from slices previously heat-inactivated before addition of the drug.

RESULTS

Urinary Elimination of Dormison by Dogs Following Oral Administration.—Adult dogs, maintained in individual metabolism cages, were given the drug in a capsule and in one single dose. Consecutive twenty-four-hour urine samples were collected, the volumes recorded, and each sample filtered. From the filtered sample, a 75-ml. aliquot was analyzed for the drug using the procedure described above. The results are recorded in Table I.

It is noted that the per cent of the total dose excreted in the urine ranged from 0.5 to 1.7 during the first twenty-four hours. In one experiment (dog IV), in which daily collections were analyzed for a three-day period following a single oral dose, only 3.3% of the total dose was recovered.

To make certain that this low level of elimination was real, and not due to loss of drug by evaporation during collection, an experiment was carried out on a pentobarbital-treated dog who had received Dormison orally immediately before administering the barbiturate. A catheter was put into place and the urine collected for two consecutive seven-hour periods. Analysis of the urine revealed a total recovery of 1.7% of the total dose during the fourteen-hour period, a recovery of the same order of magnitude obtained with nonanesthetized dogs maintained in metabolism cages.

Blood Levels of Dormison in the Dog Following Intravenous Administration of the Drug.—Following the intravenous administration of the drug,

venous blood samples were obtained at several intervals. Table II summarizes the data obtained with two dogs. Within the first ten minutes after drug administration, the blood level is already low. Assuming that the blood makes up 9.6% of the body weight (3), 20% of the administered dose was accounted for in the blood one and one-half to ten minutes after administration of the drug; by twenty to twenty-five minutes only 12-14% was present.

TABLE II.—DORMISON BLOOD LEVELS IN DOGS FOLLOWING INTRAVENOUS ADMINISTRATION

Animal No.	Body Wt., Kg.	Dose, Mg./Kg. Body Wt.	Collection Period, Min.	Total Dose in Blood,[a] %
V	10.5	200	1.5	21.6
			12.0	20.0
			20.0	11.7
VI	10.7	200	10.0	21.5
			25.0	14.0
			120.0	None detected

[a] Total blood volume assumed equivalent to 9.6% of body weight (3).

Concentration of Drug in Tissues of the Rat.—Because it was not possible, due to sensitivity limits of the method, to analyze the various organs of the dog for the drug, a study of the fate of the drug was carried out in the tissues of the rat. For this purpose the whole brain and spleen, both kidneys, the entire liver, and representative samples of skeletal muscle and adipose tissue were separately studied. The drug was administered by gavage (800 mg.-Kg. body weight) and when the animals exhibited signs of sedation, they were sacrificed and the tissues removed.

Table III reveals that for all tissues, with the exception of liver, less than 1% of the total administered dose was present; in the liver approximately 6% was found. However, if an approximate estimate of the amount in the total muscle and adipose tissue is made, it appears that a significant amount is present in both these tissues. In an experiment in which the drug was permitted to "wear off," as evidenced by the disappearance of the hypnotic effect of the drug, no measurable drug was found in any organ including the liver.

In Vitro Inactivation of Dormison by Tissue Slices.—Because the data indicated that only small amounts of the drug were eliminated in the urine, that the blood levels decreased rapidly following intravenous administration of the drug, and that little of the drug remained in the tissues, it appeared likely that the compound was rapidly metabolized by the tissues and/or the blood, or possibly eliminated through the lungs.

Experiments revealed no evidence of Dormison inactivation by either whole blood or plasma. Therefore, an examination of the effect of certain tissue slices (liver, kidney, and brain) on Dormison was made. Table IV presents typical data from one of several experiments relating time of incubation to the per cent of the drug inactivated during a given time period. It is evident that each of these tissues can metabolize the drug rapidly, *in vitro*. The liver and kidney are more effective than the brain in this respect. That the inactivation probably involves enzymatic processes is indicated by the fact that subjecting the tissue slices to 90° in glycine buffer for ten minutes before adding the drug almost completely abolishes the ability of the tissue to inactivate the compound.[1]

TABLE III.—DORMISON CONTENT OF RAT TISSUES

Tissue	% Total Dose Found
Brain (total)	0.6
Spleen (total)	0.3
Kidneys (both)	0.6
Fat[a]	Approx. 12.0
Muscle[b]	Approx. 4.0
Liver (total)	6.2

[a] Calculated for fat in entire body assuming 20% of body weight as adipose tissue (4).
[b] Calculated for muscle in entire body assuming 40% body weight as muscle (5).

TABLE IV.—INACTIVATION OF DORMISON BY LIVER, KIDNEY, AND BRAIN SLICES

Incubation Time, Min.	% Drug Inactivated/200 Mg. Tissues[a]		
	Liver	Kidney	Brain
0	0	1.5	3.5
15	14	28.5	7.5
30	36	35.5	17.5
90	43	46.5	17.5

[a] Values corrected for heat-inactivated control.

SUMMARY

1. Methods are described for the estimation of Dormison (3-methyl-pentyne-ol-3, methyl parafynol) in biological fluids and tissues.

2. A major step in the metabolism of Dormison is the destruction of the ethinyl group which occurs very rapidly in the dog as revealed by the rapid decline in blood level, low level of elimination in the urine, and the *in vitro* demonstration, in rat tissues, of the disappearance of this structural group in the presence of tissue slices.

3. No evidence of storage or accumulation of the drug in the tissues was found.

REFERENCES

(1) Margolin, S., Perlman, P., and Villani, F., *Science*, **114**, 384(1951).
(2) Siggia, S., "Quantitative Organic Analysis via Functional Groups," John Wiley & Sons, New York, 1949, p. 49.
(3) Dukes, H. H., "The Physiology of Domestic Animals," 5th ed., Comstock Publishing Co., Ithaca, N. Y., 1943, p. 55.
(4) Banks, A., Hilditch, T. P., and Jones, E. C., *Biochem. J.*, **27**, 1375(1933).
(5) Hawk, P. B., Oser, B. L., and Summerson, W. H., "Practical Physiological Chemistry," 12th ed., Blakiston Co., Philadelphia, 1947, p. 231.

[1] Studies on the effects of Dormison on the metabolism of tissue slices *in vitro* will be reported in a separate paper.

Hydrolytic Stability of Saccharin*

By OLIVER DeGARMO, GEORGE W. ASHWORTH, CHARLES M. EAKER, and RALPH H. MUNCH

The influence of *p*H and temperature on the hydrolysis of saccharin was studied. An ultraviolet absorption method was used to determine the amount of saccharin decomposed. The limit of accuracy of the determination represents 1 per cent decomposition. It was found that for all practical purposes saccharin in aqueous buffered solutions of *p*H 3.3, 7.0, and 8.0 is unaffected by heating at temperatures of 100, 125, and 150° for one hour.

SACCHARIN has been used for many years as a sweetening agent in pharmaceutical products, in beverages, and in various foods. It is also used in place of sugar by diabetics. Saccharin has approximately 300–500 times the sweetening power of sugar and is harmless in quantities used for sweetening (1). However, it has no food value.

The stability of saccharin in aqueous solutions has been studied previously (2, 3). These workers concluded that the sodium salt of saccharin (soluble saccharin) is very slowly hydrolyzed in hot solutions. For example, heating a 0.001 *M* solution of the sodium salt at 230° for two hours resulted in a loss of only 6 per cent of the saccharin.

By comparison with the sweetness of sugar solutions no hydrolysis of soluble saccharin could be detected after heating at 100° (2). These studies did show that in strongly acid solution saccharin was hydrolyzed appreciably.

Absorption spectra were used as the analytical method in one study (2), while in the second (3) saccharin was determined by titration with silver nitrate. In the latter procedure a correction factor of +3 per cent was necessary.

In recent months there have been observed numerous statements implying that saccharin is lost during food processing operations. The *p*H of most food products falls between the extremes of 3–8 (4). Fruit and fruit products are usually *p*H 3–4, vegetables and meat products have a *p*H range of 5–7, while baked goods may have a *p*H as high as 8. Maximum temperatures encountered in food processing rarely exceed 150°. The temperatures of properly browned crusts of baked products do not exceed 150°, while the temperature attained within most doughs during baking is approximately that of boiling water (5). Processing temperatures in canning operations are below 150°. It is the purpose of this paper to study the stability of aqueous saccharin solutions under *p*H and temperature conditions encounted in food processing.

The hydrolysis products of saccharin are *o*-sulfamyl benzoic acid (alkaline hydrolysis) and (ammonium-*o*-sulfo)benzoic acid (acid hydrolysis). Pure samples of these acids as well as saccharin were prepared and their ultraviolet absorption curves obtained. These curves are sufficiently different so that the ultraviolet absorption can be used to determine the amount of decomposition of saccharin in aqueous solution.

EXPERIMENTAL[1]

Purification of Saccharin.—Commercial grade saccharin was recrystallized twice from acetone. M. p. 228.8 to 229.7°.

Preparation of *o*-Sulfamyl Benzoic Acid.—This material was prepared essentially as described earlier (3). The product from the alkaline hydrolysis of saccharin was twice crystallized from absolute ethanol, m.p. 155.8 to 156.8°; reported (3) 157° (uncorr.).

Preparation of (Ammonium-*o*-Sulfo)Benzoic Acid.—This product was prepared from saccharin by a slight modification of a procedure described earlier (6). Saccharin (188 Gm.) was gradually added over a one-hour and forty-minute period to a refluxing solution of 79 ml. of hydrochloric acid in 282 ml. of water. The solution was refluxed for two hours after the saccharin had all been added, filtered hot, and cooled to 2°, after which the product was filtered and washed with ice water. The filtrates were concentrated under vacuum to recover a second crop. The combined crops (182.9 Gm.) were recrystallized from 180 ml. of water to yield 160 Gm. of product, m. p. 268–270°; reported (3) 265° (uncorr.).

Determination of Ultraviolet Absorption Curves.—Ultraviolet absorption curves for saccharin, *o*-sulfamyl benzoic acid and (ammonium-*o*-sulfo)benzoic acid were obtained in 0.1 *N* sodium hydroxide, and in solutions buffered at 8, 6, and 3 *p*H, as well as in 0.1 *N* hydrochloric acid using a Cary recording spectrophotometer. Inspection of the curves showed that the best analytical results could be obtained in 0.1 *N* sodium hydroxide solution.

Figure 1 shows the curves obtained for the three compounds in this solvent. It is only in 0.1 *N* sodium hydroxide solution that all three compounds have their absorption maxima at approximately the same wave length; this is the reason for making measurements under these conditions.

* Received August 31, 1951, from the Organic Chemicals Division Research Laboratories, Monsanto Chemical Co., St. Louis, Mo.
Presented to the Scientific Section, A. PH. A., Buffalo meeting, August, 1951.

[1] All melting points are corrected.

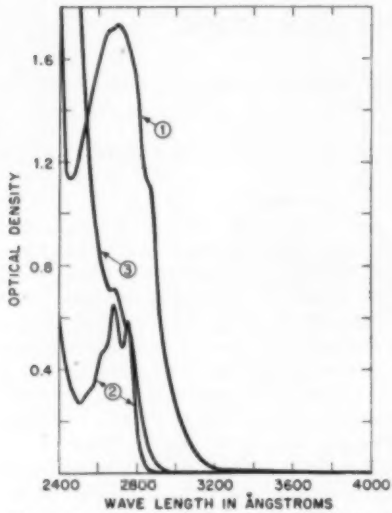

Fig. 1.—0.01 % Solutions in 0.1 *N* NaOH. 2.0 cm. cell thickness. (1) Saccharin, (2) (Ammonium *o*-sulfo) benzoic acid, (3) *o*-Sulfamyl benzoic acid.

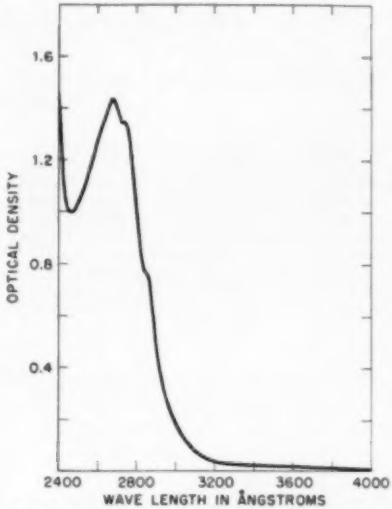

Fig. 2.—0.0375 % Saccharin in 0.1 *N* NaOH. 0.5 cm. cell thickness. Measurement after heating 0.375 % saccharin solution in water one hour at 150°.

(Ammonium *o*-sulfo)benzoic acid has a sharp maximum at 2673 Å. with a molecular absorption coefficient of 7140. The *o*-sulfamyl benzoic acid does not have as sharp an absorption peak as the (ammonium *o*-sulfo)benzoic acid; however, 2673 Å. is very close to the maximum with a molecular absorption coefficient of 7100. Saccharin has a rather broad absorption peak with a molecular absorption coefficient of 15,700 at 2673 Å. Since the two possible decomposition products have essentially the same molecular absorption coefficient, and since this value is considerably less than for saccharin itself, it is possible to determine the amount of decomposition of saccharin without knowing how much of either decomposition product is formed. The over-all accuracy of the method is about ±1%.

TABLE I

Solvent	*p*H of Soln.	% Decomposition After One Hour at: 100°	125°	150°
Water	2.0[a]	2.9	8.5	18.6[b]
Buffer	3.3	0	1.0	1.9
Buffer	7.0	0.3	0.3	1.6
Buffer	8.0	0	0	0

[a] *p*H of 0.35% solution of purified saccharin in water.
[b] The absorption curve for this solution is given in Fig. 2.

Hydrolysis Studies.—Samples of purified saccharin weighing approximately 0.8500 Gm. were transferred to 250-ml. volumetric flasks. The theoretical volume of 0.5 *N* sodium hydroxide solution was added, and the resulting solution was diluted to the mark with Clark and Lubs buffer of the desired *p*H. A 25-ml. sample was pipetted into a Pyrex tube, the tube was sealed and then heated in an oil bath at the designated temperature for one hour. The ampuls were opened, and the contents transferred to a 250-ml. volumetric flask and made up to volume with 0.1 *N* sodium hydroxide. Absorption curves were determined, and the amount of decomposition was calculated. Results obtained are tabulated in Table I.

DISCUSSION

The limit of accuracy of the ultraviolet absorption method used represents 1 per cent decomposition. With this limitation in mind, the results obtained confirm those previously reported (2, 3). The extent of hydrolysis found in the *p*H range of 3.3 to 8.0 and 100° to 150° indicates that for practical purposes saccharin is stable. Figure 2 gives further evidence that hydrolysis of saccharin at *p*H 2 forms (ammonium-*o*-sulfo)benzoic acid.

REFERENCES

(1) Lehman, A. J., *Assoc. Food & Drug Officials U. S. Quart. Bull.*, **14**, 82(1950).
(2) Taufel, K., and Naton, J., *Z. angew. Chem.*, **39**, 224 (1926).
(3) Taufel, K., Wagner, C., and Preisz, W., *Z. Elektrochem.*, **34**, 281(1928).
(4) Pilcher, R. W., "The Canned Food Reference Manual," 3rd ed., American Can Co., New York, 1947, pp. 522–524.
(5) Barackman, R. A., and Bell, R. N., *Cereal Chem.*, **15**, 841(1938).
(6) Gilman, H., "Organic Syntheses," Coll. Vol. I, John Wiley & Sons, New York, 1941, p. 14.

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Studies of the Effects of Season, Temperature, and Plant Age on Glycoside Production in *Digitalis purpurea*[*,†]

By D. P. N. TSAO[‡] and H. W. YOUNGKEN, Jr.[§]

A study of several growth conditions indicated that the optimum glycoside production in *Digitalis purpurea* takes place in young actively growing first-year plants rather than in older one-year plants in which metabolic breakdown is dominant. Seasonal factors other than temperature changes exert some influence in the field but do not significantly affect glycoside production under controlled greenhouse conditions.

MANY FACTORS undoubtedly affect the production of glycosides in *Digitalis purpurea*. It has long been thought that among these the season of growth, critical temperature changes and the age of plants are among the most influential factors. But opinions vary as to the relative importance of each of these during glycoside production. Sharp and Lancaster (1) have reported that digitalis plants grown in England should be collected during June or July for optimum glycoside yields. Hepler, *et al.* (2), on the other hand, have shown that greater yields of glycosides were present in digitalis plants that had been collected in October. Plants used in the latter study were grown in New Hampshire. Imoto (3) has further reiterated that the season of digitalis growth has much to do with its glycoside synthesis. He has also suggested that the age of the plant might have some correlation with proper time for optimum glycoside production.

The purpose of this study has been to investigate these factors under more carefully controlled field and greenhouse culture methods and to ascertain the comparative importance of each as it may bear influence on proper time for digitalis leaf collection. .

MATERIALS AND METHODS

Digitalis plants used for this study were developed under carefully controlled field and greenhouse conditions. All seedlings and mature plants were grown from known strains of *Digitalis purpurea*, Linné, which contained flowers of mixed colors, i. e., purple, white, lavender. Seeds were germinated in the greenhouse and seedlings were transplanted from culture flats directly into the field or into culture solutions prior to the study of growth and glycoside responses. Usually the leaf blades of seedlings measured about 6 cm. in length and roots measured up to 10 cm. in length at the time of transplanting.

Experimental plots for field studies were arranged in the Drug Plant Gardens, University of Washington. An examination of the soils in each plot was made and found to be sufficiently uniform in nitrogen, phosphorus, and potassium for use. Soil *p*H ranged from 6.0 to 6.5.

Digitalis plants for hydroponic study were developed from seed material in the same manner as above. From 60- to 80-day-old seedlings were transplanted from flats into black 1.5-L. wide-mouth bottles filled with culture solution. Two holes were made into each metal bottle cap which had previously been coated with paraffin and one seedling was inserted into one hole and held firmly with cotton. The second aperture was loosely plugged with cotton for sufficient aeration of the culture solution within. Water tables were kept at approximately 2 in. below the bottle cap. It was found that this provision for culture aeration was adequate and good plant growth resulted.

A modified Hoagland's solution (4) was employed for digitalis growth in bottle feedings. This consisted of the following formula in which chemically pure reagents were dissolved in distilled water:

$Ca(NO_3)_2$	0.005 *M*
KNO_3	0.005 *M*
$MgSO_4$	0.002 *M*
KH_2PO_4	0.001 *M*
Supplementary solution	1 cc./L. of soln.
Ferric tartrate 0.5%	1 cc./L. of soln.

The formula of a supplementary solution that was supplied is as follows:

H_3BO_3	2.66 Gm.
$MnCl_2 \cdot 4H_2O$	1.81 Gm.
$ZnCl_2$	0.11 Gm.
$CuCl_2 \cdot 2H_2O$	0.05 Gm.
Distilled water, sufficient quantity to make	1,000 cc.

Ferric tartrate 0.5% was occasionally added to the culture solution, or it was sprayed onto the leaves whenever indications of iron deficiency were observed. Fresh solutions were employed each week during the study. The *p*H of these solutions was maintained at approximately 5.0 to 5.5.

* Received August 31, 1951, from the College of Pharmacy, University of Washington, Seattle.
Presented to the Scientific Section, A. PH. A., Buffalo meeting, August, 1951.

† This paper is based on part I of a thesis presented to the Graduate School, University of Washington, by Daniel P. N. Tsao in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Growth measurements of all plants were recorded every week. Representative leaves were selected from each plant and the length of blade and petiole, the length and width of blade, and the length of roots were recorded in centimeters. The successive measurements were compared for the rate of plant growth.

At the end of each experimental growth period all plants were harvested and leaves and roots were separated. The weights of fresh leaves and roots of each group were recorded separately. Plant material was then transferred immediately into a circulating hot air dryer and dried at 30° C. Dried weights and moisture content were measured, and dried leaves were then ground to a No. 60 powder by means of an Abbe electric grinder. This material was then stored in air-tight containers for glycoside analysis.

Digitalis tinctures were prepared according to the U. S. P. XIV method (5). The total glycoside was assayed by using a modified Knudson-Dresbach method (6). The transmission was compared at 525 mμ in a 1-cm. cell by the use of a Beckman Model DU Photoelectric Spectrophotometer. A standard curve was constructed from a tincture prepared with U. S. P. Digitalis Reference Standard. Unknown tinctures were then compared with the Standard Digitalis Reference Tincture and the glycoside yields were expressed in per cent potency of the latter reference standard, expressed as 100 %.

Several tinctures were also assayed by the established U. S. P. XIV (5) biological procedure for digitalis tincture. This method was modified by providing a continuous dose injection into the alar vein of the pigeon rather than employing a five-minute-interval injection technique. It was found that a continuous injection technique was more convenient and resulted in a decrease in the incidence of blood clotting in the vein. A Merck laboratory infusion pump into which a 20-cc. standardized syringe had been inserted was employed for continuous infusion. Pentobarbital sodium (40 mg./Kg.) provided more convenient and suitable anesthesia than did ether. All tinctures were then compared in activity with that of a U. S. P. Reference Standard. The standard error of the mean (7) was calculated by the conventional method.

TABLE I.—TEMPERATURE VARIATIONS DURING FIELD GROWTH STUDIES OF DIGITALIS

Month	Average Day Temperature, °F.	Average Night Temperature, °F.
June	74.2	53.2
July	72.7	53.8
August	70.8	56.4
September	69.0	49.1
October	60.4	43.8
November	48.8	38.5
December	43.7	32.5
January	38.7	26.5

EXPERIMENTAL

Field Study.—Two hundred and four digitalis seedlings were transplanted into the field during March of 1948. The average temperature in the field during the period of study was carefully noted. The seasonal variability of temperature throughout the experiment is shown in Table I. Representative leaves from each plant growing in the field were collected periodically from June 20, 1948, to January 19, 1949, and at the end of 87, 110, 133, 141, 153, 184, 215, 284, and 303 days of growth. These collections were then assayed for total glycosides by the methods previously described. Results are shown in Table II and Fig. 1.

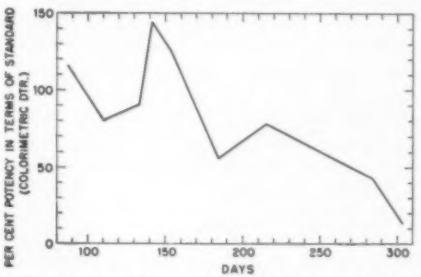

Fig. 1.—Total glycoside production at periodical intervals in the first-year digitalis growing in the field (March to January).

The highest glycoside yield (144.50%) was found to occur in leaves of plants collected during August or at the end of 141 days of growth; the lowest (14%) occurred in those collected in January or at the end of 303 days of growth. Many leaves at the latter time were frozen and some were yellow in color. Leaves in the other collections were normal in every aspect and growth was uniform in all ex-

TABLE II.—GLYCOSIDE YIELDS OF FIRST-YEAR DIGITALIS PLANTS GROWING IN THE FIELD. DATA FROM PERIODIC COLLECTIONS

Age of Plants, Days	Time of Collection	Colorimetric Assay (% Potency in Terms of U. S. P. Ref. Std.)	No. of Pigeons	Biological Assay (% Potency in Terms of U. S. P. Ref. Std.)
87	June 29, 1948	116.00		
110	July 12	80.50		
133	August 4	90.50		
141	August 12	144.50	4	127.00 ± 9.00
153	August 24	126.00		
184	September 24	56.00		
215	October 25	78.00		
284	January 2, 1949	43.00	4	40.12 ± 3.90
303	January 19	14.00		

perimental plots employed for the field study. Glycoside yields were decreased greatly in samples which were harvested after August (see Table II and Fig. 1). Leaf material collected in August and January was also assayed biologically and the results are shown in Table II. These results were similar to those obtained by the colorimetric method and again indicated that in this locality glycoside production among digitalis plants grown in the field was greatest during the middle of August or when plants had reached between 133 and 153 days of growth. Younger and older plants generally showed less glycoside production. Collections made in June, while not as high in glycoside content as those in August, were considered to be sufficiently good for drug use.

Hydroponic Culture Study.—For this study digitalis seedlings of different ages (see Table III) and size measurements were arranged into five groups, i. e., groups A, B, C, D, and E. All seedlings were carefully washed and then transplanted from soil medium into 1.5-L. bottles according to the method previously described. Greenhouse temperatures were maintained between 70 to 75° F. Each group, with the exception of those in group E, contained not less than 10 plants, and there were 85 plants in group E. All groups were examined at the end of forty-one to forty-nine days in culture solution. Plants in group E were also examined for glycosides at the end of twenty-one, twenty-eight, thirty-five, and forty-two days. Plants grew vigorously in the culture solution and exhibited no toxic effects. Growth measurements were made every week. The rate of growth among these five groups did not show any significant differences.

Leaves from plants in groups A, B, C, and D were collected at the end of the sixth or seventh week of growth in culture and were assayed by both colorimetric and biological methods; those from plants of group E were collected at the end of the third, fourth, fifth, and sixth weeks of growth and these were assayed in the same manner.

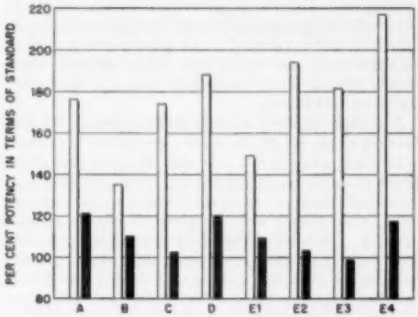

Fig. 2.—Glycoside production in digitalis grown in hydroponic culture. Blank bar—colorimetric assay; solid bar—biological assay. (*A*) Aug.–Sept.; (*B*) Sept.–Oct.; (*C*) Oct.–Nov.; (*D*) Jan.–Mar.; (*E*) April–May.

A difference in total glycoside production among groups was apparent following analyses by the colorimetric method (see Table IV and Fig. 2). Plants of groups A and D, which were grown in the greenhouse during August and early September and late January to the middle of March, respectively, showed higher glycoside yields than those of groups B and C, which were grown under the same conditions but during late September to November. The glycoside yields from leaf material of plants grown during August to September and January to March was approximately 176–188%, whereas in plants grown under the same conditions during September to November, yields were from 135 to 141%. These results were not consistent however, with the probability that glycoside production is in direct association with seasonable factors such as was indicated during field observations. Otherwise, one would expect lower yields from leaf material grown during January to March even under greenhouse

TABLE III.—AGE AND SEASON FACTORS IN DIGITALIS PLANTS GROWN IN HYDROPONIC CULTURE SOLUTION

Group	No. of Plants	Age of Plants When Transplanted, Days	Period in Distilled Water, Days	Period in Culture Soln., Days	Total Age of Exptl. Plants When Harvested, Days	Season
A	10	80	14	42	136	Aug.–Sept.
B	12	50	6	49	105	Sept.–Oct.
C	10	58	7	42	107	Oct.–Nov.
D	20	77	10	41	128	Jan.–Mar.
E	85	78	7	21, 28	106, 113	Apr.–May
				35, 42	120, 127	

TABLE IV.—GLYCOSIDE ANALYSIS OF DIGITALIS LEAVES FROM PLANTS GROWN IN CULTURE SOLUTION

Group	Age in Days	Colorimetric Assay (% Potency in Terms of U. S. P. Ref. Std.)	Biological Assay	
			No. of Pigeons	% Potency in Terms of U. S. P. Ref. Std.
A	136	176.00	4	120.20 ± 8.65
B	105	135.00	5	110.06 ± 6.63
C	107	135.00	5	102.24 ± 11.89
D	128	188.00	4	119.65 ± 5.50
E	106	149.00	4	109.19 ± 9.70
E	113	164.00	6	.....
E	120	181.00	4	.....
E	127	217.00	4	117.21 ± 7.50

conditions. The major difference among plants in experimental groups grown in culture solution was their age when collected. All plants which yielded high glycoside content (groups A and D) were from twenty-two to thirty days older than those producing lesser quantities.

A periodic study was then made of glycoside production in plants of the same age (group E) grown in culture solution for a period of forty-two days. These plants were grown during April and May and were examined at the end of twenty-one, twenty-eight, thirty-five, and forty-two days in culture solution. Results of chemical and biological assays (see Table IV) indicated that there was a general increase in glycoside production during the forty-two-day growth period. The highest glycoside production occurred during the last week of growth, or when plants were approximately 127 days old. This checked closely with the results noted in plants of groups A and D, which were 136 and 128 days old, respectively, and which gave highest glycoside yields. It further substantiated the fact that digitalis plants grown under these conditions produced better yields of glycosides when they had progressed beyond about 120 days of growth, regardless of the season.

Inasmuch as the temperatures were kept constant, i. e., within 70 to 75° F., during the greenhouse studies, it was apparent that even within this range variations did occur in glycoside production. It was concluded that among the three factors studied (season, temperature, and age), that of age seemed most important as a criterion for the optimum production of total glycosides. During first-year growth in culture solution, plants which were from 120 to 136 days old gave best yields, whereas those from 141 to 156 days of growth in the field yielded optimum total glycosides.

Comparative Results of Chemical and Biological Assay Data.—Although the results of the biological analyses of digitalis glycosides in leaves of plants grown in the field and in culture solution followed the general trend of those found from chemical assay (see Table IV) it was obvious that the latter gave much higher and more consistent results. In fact, U. S. P. pigeon assays, *per se*, of culture solution material actually did not show differences beyond the tolerance range of ±20% for testing of this kind. It was therefore thought that certain nutrient elements in the Hoagland solution formula might be responsible for stimulating the production of noncardiotonic glycosides during digitalis growth in this culture. These glycosides would respond to the chemical assay but not to the biological assay. It is reasonable to suspect that the same factor might take place in leaf samples from digitalis field growth. Chen (8) has stated that the proper number and kind of sugar molecules attached to the aglycone portion of the digitalis glycoside molecule governs the cardiotonic activity within a critical amount; further, that activity is lessened if more or less than the proper number of sugar molecules are attached. However, free sugars do not react under the same chemical testing methods as employed here. No further study was made to ascertain the reason for such divergent results as observed during this study.

SUMMARY

Largest quantities of glycosides were found to be present in leaves of 140- to 156-day-old *Digitalis purpurea* plants grown in the field. A periodic study of seasonal variations and glycoside production indicated that August was the optimum season for leaf harvest of first-year plants growing in the Pacific Northwest.

When grown under carefully controlled greenhouse and culture solution methods, actively metabolizing plants formed glycosides in largest amounts at approximately the same age (120 to 136 days of growth) regardless of season and temperature variations.

Notable differences between results of glycoside assays by the U. S. P. XIV colorimetric and pigeon infusion methods have been cited.

REFERENCES

(1) Sharp, J. G., and Lancaster, J., *Pharm. J.*, **86**, 102 (1911).
(2) Hepler, J. R., Ackerman, W. T., and French, B., *Proc. Am. Soc. Hort. Sci.*, **40**, 557(1942).
(3) Imoto, M., *J. Pharm. Soc. Japan*, **59**, 384(1939).
(4) Meyer, B. S., and Anderson, D. B., "Plant Physiology," 8th printing, D. Van Nostrand, New York, 1939.
(5) "United States Pharmacopeia," Fourteenth Revision, Mack Publishing Co., Easton, Pa., 1950, p. 179.
(6) Bell, F. K., and Krantz, J. C., *J. Pharmacol. Exptl. Therap.*, **83**, 213(1945).
(7) Hall, M. F., "The Presentation of Medical Statistics," Overbeck, Ann Arbor, Mich., 1946.
(8) Chen, K. K., *Ann. Rev. Physiol.*, **1**, 677(1945).

An *in Vitro* Method for Assaying the Biological Activity of Tyrothricin*,†

By A. KATHRINE MILLER, CLARA MATT, and JOSEPH L. CIMINERA

The U. S. P. tyrothricin assay has been modified by using a young seed culture and by using growth response rather than time to determine the test incubation period. The resulting assay saves time and materials and is more precise than the currently used tests. It is completed within a working day and is so designed that a simple statistical formula can be applied to obtain 95 per cent confidence limits for each potency figure.

TYROTHRICIN is an antibiotic agent produced by *Bacillus brevis;* it was first isolated by Dubos in 1939 (1). Although several methods of assay have been reported (2, 3), the ones commonly used to determine the biological activity of tyrothricin are modifications of the so-called "Dubos assay." This is the tube dilution test in which replicates of two to ten dilutions of both tyrothricin standard and the test preparation are made for each assay. As described in the Fourteenth Revision of the United States Pharmacopeia (4), the culture medium for this type test is enriched with serum albumin, seeded with a twenty-four-hour culture of the group D hemolytic streptococcus H69D_k, and the test is read after overnight incubation. Replicate tubes in such a test often may show wide variations in turbidity and the reproducibility of potency estimates is not always satisfactory. In an attempt to improve the assay, a test method has been developed that appears to be valuable because it is more precise and because it saves time, effort, and materials. It is possible to eliminate the daily preparation of both standard dilutions and seed culture by making them in bulk once every two weeks. The range of turbidity variation among replicate tubes has been considerably decreased and the recovery of known tyrothricin concentrations is at least as satisfactory as with the Dubos assay. Moreover, the test has been designed so that a simple statistical formula can be applied to obtain 95 per cent confidence limits for each potency figure.

TEST MATERIALS AND MEDIA

Propylene Glycol-Alcohol Diluting Fluid.—Mix equal quantities by volume of propylene glycol (U. S. P.) and distilled water. Sterilize by holding at 100° for twenty minutes. To 87.5 ml. of this 50% propylene glycol, add 12.5 ml. of 95% ethyl alcohol and mix thoroughly.

Bovine Serum Albumin Solution.—Prepare a 5% albumin solution by dissolving Armour's Fraction V bovine albumin in distilled water with the aid of mechanical stirring. If necessary, adjust the *p*H to between 6.8 and 7.2 with sodium hydroxide. Sterilize by Seitz filtration and store at 5° until used.

Assay Base Medium.—

Bacto-tryptone	10.0 Gm.
$Na_2HPO_4 \cdot 12H_2O$	3.6 Gm.
Dextrose, U. S. P.	5.0 Gm.
Distilled H_2O, *q. s.*	1,000 ml.
*p*H	6.2

Inoculum Medium.—Although it is believed that any similar medium may be used, the formula used in this laboratory follows:

Lean veal, ground	1.5 lb.
Neo-peptone, Difco	20.0 Gm.
NaCl	5.0 Gm.
Distilled H_2O	1,000 ml.
*p*H	7.5

Add cold distilled water to veal, mix thoroughly, and allow to stand overnight at +5°. Heat to 58°, allow to stand one and one-half hours, bring to boil, and boil fifteen minutes. Strain juice through cotton and *q. s.* to 1,000 ml. Add peptone and NaCl, bring to a boil; adjust *p*H to 7.8; boil fifteen minutes and again adjust *p*H to 7.8. Filter through sterile paper into sterile bottles. Sterilize by exposing to streaming steam for ninety minutes. Incubate forty-eight hours to check sterility.

Test Organism Stock Culture.—A hemolytic streptococcus, Lancefield group D, strain No. H69D_k (American Type Culture Collection No. 9854) is carried on 5% blood agar slants. The culture is transferred every two or three weeks and, following forty-eight-hour incubation at 37°, the slants are stored at 5°.

Preparation of the Inoculum.—Sufficient inoculum for use over a two-week period may be prepared at one time. An eighteen-hour broth culture of the test organism is made by transferring from a blood agar slant to a tube of inoculum medium, followed by incubation overnight at 37°. One-tenth milliliter of this broth culture is used to inoculate 5 ml. of inoculum medium in a colorimeter tube. The initial turbidity is read and recorded, and the tube is incubated in a 37° water bath. The developing turbidity of the inoculated tube is followed until the growth curve as plotted on plain graph paper indicates the culture definitely is in the logarithmic phase of growth. In

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† Presented in part at the 51st General Meeting of the Society of American Bacteriologists, Chicago, May, 1951.

our hands, this occurs approximately three to four hours after inoculation and is represented by a Klett-Summerson photoelectric colorimeter reading of 35–45 when the machine is set at zero with uninoculated medium and the No. 54 green filter is used. At this time 4 ml. of this culture is transferred from the colorimeter tube to 100 ml. of inoculum medium that has been warmed to 37°. After thorough mixing, 5 ml. of this inoculated culture is transferred aseptically to a sterile colorimeter tube so that the growth of this second transfer also can be followed turbidimetrically. The initial turbidity of this tube is recorded and incubation is continued at 37°. When the plotted curve of this second culture shows the beginning of the logarithmic phase (a turbidity reading of fifteen to twenty-five after two to two and one-half hours' incubation), the bottle of inoculum is transferred immediately to a +5° cold vault where it is stored for use over the following two-week period.

Tyrothricin Reference Standard Stock Solution.—Weigh accurately 50 mg. of U. S. P. Tyrothricin Reference Standard and transfer it, with the aid of 95% ethyl alcohol, to a 50-ml. volumetric flask. Dilute to volume with ethyl alcohol and mix thoroughly. This 1 mg./ml. tyrothricin solution is stored at 5° in a tightly stoppered bottle and may be used indefinitely.

Preparation of the Tyrothricin Standard Dilution.—Using volumetric flasks throughout, the 1 mg./ml. tyrothricin reference standard is diluted with ethyl alcohol to a concentration of 0.05 mg./ml. This may be done conveniently by making a 1:10 dilution followed by a 1:2 dilution in 10-ml. volumetric flasks. The 0.05 mg./ml. solution then is diluted 1:100 with the propylene glycol-alcohol diluting fluid to make a solution containing 0.0005 mg./ml. From this master solution, the four final dilutions are made by transferring carefully to 50-ml. volumetric flasks 22.5 ml., 15 ml., 10 ml., and 6.6 ml. These are diluted to volume with the propylene glycol-alcohol diluting fluid and result in 0.000225, 0.00015, 0.0001, and 0.000066 mg. tyrothricin per ml., respectively.

These four solutions are used in the daily preparation of the standard curve and will result in a final concentration of 9, 6, 4, and 2.6 milligamma of tyrothricin per ml. of culture medium. They should be labeled appropriately and can be stored in the ice box for use over at least a two-week period. It should be noted that these concentrations are equally spaced on a logarithmic scale and vary by a factor of 1.5.

Preparation of Glassware.—As emphasized by Reedy and Wolfson (3), it is important that all glassware be cleaned chemically. In this laboratory glass-capped tubes now are used. If cotton-plugged tubes are used, they should be placed plug side down during autoclaving so that they remain free of any cotton oil that may come from the plug during sterilization.

PERFORMANCE OF THE TEST

The Standard Curve.—The four dilutions of tyrothricin used to prepare the standard curve are removed from the ice box and allowed to reach room temperature. Using 2-ml. pipettes, 0.2 ml. of each dilution is placed in each of six glass-capped test tubes. Two-tenths milliliter of the highest and of the lowest concentrations also is transferred to sterile colorimeter tubes. These two tubes are used as check tubes during the test incubation period.

Preparation of Test or Unknown Materials.—Only a single concentration of the test sample need be prepared. On the basis of labeled potency, materials to be assayed are diluted to 0.05 mg. tyrothricin per ml. with 95% ethyl alcohol. These further are diluted with the propylene glycol-alcohol diluting fluid, in volumetric flasks, to 0.000125 mg. tyrothricin per ml. Using a 2.0-ml. pipette, 0.2 ml. of this final test material dilution is placed in each of six glass-capped test tubes. The final concentration of tyrothricin per ml. of culture medium will be 5 milligamma if the test preparation is 100% of its labeled potency.

Preparation of Inoculated Assay Medium.—Immediately before seeding the test, the assay medium, bovine serum albumin, and inoculum are removed from the refrigerator and are mixed so as to be used at refrigerator temperature. For every 80 ml. of assay medium, 20 ml. of bovine serum albumin and 2 ml. of inoculum are added. After thorough mixing, 5 ml. of the inoculated medium is added to each tube. In this laboratory a Brewer automatic pipetting machine is used for this purpose.

The arrangement of the tubes in the rack and the order in which they are seeded and subsequently handled is that suggested in Fisher and Charney (5) and is shown on Fig. 1. The replicate tubes are placed in parallel rows, the standard tubes first, followed by the samples to be assayed. In addition, at least three seeded check tubes are included; these are Klett tubes, one of which is used as a growth control and contains, in addition to the medium, only the propylene glycol-alcohol diluting fluid; the second contains 9 milligamma of tyrothricin; and the third contains 2.6 milligamma of tyrothricin per ml. of medium. An unseeded medium control containing the propylene glycol-alcohol diluting fluid is used as a blank to adjust the colorimeter.

The order of adding the seeded medium meanders, as is indicated by the arrows on Fig. 1. In this way any inequalities of the medium caused by possible growth of the organism during the time of seeding will be distributed evenly over all standard and unknown rows.

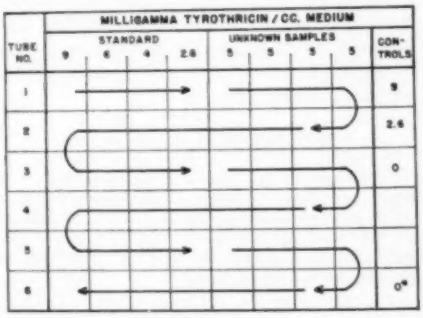

Fig. 1.—Arrangement of tubes in the rack, showing the meandering order of seeding and reading.

Immediately after seeding, the test is placed in a 37° water bath, and growth is followed by means of the check tubes. When their turbidities indicate a spread of 30–40 Klett turbidity units between the highest and lowest concentrations of tyrothricin, the response is considered to be satisfactory and further growth in the test is stopped by transferring the tubes to an ice water bath and by adding two drops of formalin to each tube. In this laboratory, the incubation time necessary to reach this point is five to five and one-half hours, and the test, therefore, can be completed within the working day.

The meandering order used for seeding is repeated not only for the addition of the formalin, but it is followed when the test turbidities are read in the colorimeter in order to equalize any progressive mechanical change which might occur and affect the readings. Average turbidity figures are used to plot a standard curve on single cycle semilogarithmic graph paper, and the potencies of the unknown solutions are read from this curve as shown in Fig. 2. The average turbidity figure of the unknown is read in terms of milligamma of tyrothricin and the percentage potency is calculated on the basis of the milligamma expected to be present per ml. of medium. The data used for Fig. 2 were obtained from an actual assay in which a tyrothricin solution was diluted to contain 80, 100, and 120% of its "labeled" potency. Recovery was 76, 96, and 119%.

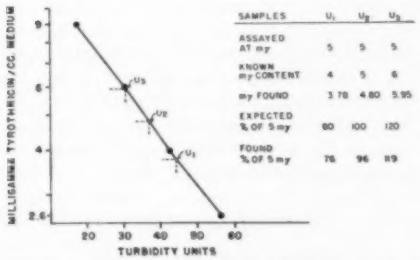

Fig. 2.—Standard curve and calculation of test samples.

Calculation of 95% confidence limits is demonstrated in Table I. The sample calculated as 96% in Fig. 2 had an average turbidity of 37; the turbidities used to calculate this average varied from 29 to 40 for a range of 11. The standard tube average turbidities immediately above and below the unknown turbidity of 37 are designated as standard high and standard low, and the ranges of their turbidities are designated as RH and RL. B is calculated by subtracting the SL from the SH. R is the sum of the three ranges. The figure 0.065 is a factor calculated for these test conditions only—that is, standard dilutions varying by a factor of 1.5, and a test using six replicate tubes at each dilution. Factors for other conditions can be calculated, and appropriate tables will be published (6). Confidence limits of 95% represent potency $\pm F$ times the potency here, ±16.9%. For potency estimates between 80 to 120%, such confidence limits have been in the range of ±8% to ±20%.

TABLE I.–CALCULATION OF 95% CONFIDENCE LIMITS

Calculated Data	Standard 9	Standard 6	Standard 4	Standard 2.6	Sample 5
Av. turbidity	17.3	30.1	42.3	56.3	37
Range	..	10	12	..	11
Potency, %	..	..	..	..	96

SH = 42.3, SL = 30.1, RH = 12, RL = 10, RU = 11.
B = SH − SL = 12.2; R = RH + RL + RU = 33

$$F = \frac{0.065\ (R)}{B} = 0.176$$

Potency $\pm F$ (Potency) = 96% ± 16.9.

DISCUSSION

The two major changes in this modified assay method as compared with Dubos-type tests such as the U. S. P. method are the use of a young seed culture and a short, variable, incubation time. A young culture was chosen for seed in the belief that its population might be more homogeneous in its sensitivity and response to tyrothricin than would a culture that is well beyond its peak growth at the time of seeding. Such a young culture proved to be more sensitive to tyrothricin than did older cultures, and the standard dilutions for the modified test contain only one-tenth as much antibiotic agent as is required to reach an end point in the U. S. P. test. Preliminary work with a young culture indicated that the effect of these concentrations of tyrothricin was not to change the rate of growth, but

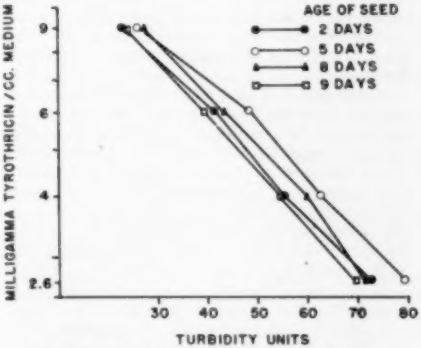

Fig. 3.—Reproducibility of standard curve.

rather to increase the lag period of the culture. Because the absolute time value of the lag phase might vary slightly from day to day and from one seed preparation to another, it seemed desirable to set the incubation time of the assay not in terms of hours, but in terms of the response of the organism to the tyrothricin standard concentrations. By adjusting the amount of young culture used as seed and the concentrations of tyrothricin used for a standard curve, it is possible to obtain a steep response curve within the time limit of a working day. That this curve is reproducible is shown in Fig. 3 in which the standard curves of a week's assays are accumulated. The same seed preparation and the same tyrothricin standard solutions were used in these tests; incubation time varied from five to five and one-half hours. This figure also illustrates that there is no apparent loss of viability in the

TABLE II.—COMPARISON OF ASSAY VALUES OBTAINED BY TWO METHODS

Test Method	Potency, % — Assay No. 1	2	3	4	5	6	Av.	Spread
U. S. P.	87	67	79	95	67	74	78	28
	119	95	88	110	85	100	100	34
	129	91	109	112	107	106	109	38
Modified	76	75	70	73	85	83	77	15
	96	101	93	94	100	100	97	8
	119	120	111	114	111	120	116	9

TABLE III.—COMPARATIVE POTENCY RESULTS OBTAINED BY TWO METHODS

Preparation Assayed	% Potency Determined by: U. S. P. Assay	Modified Assay
Extract of ointment	139	149
Nose drop	98	96
Eye wash	65	77

stored seed, nor of potency of the stored tyrothricin standard solutions. Current indications are that such tyrothricin dilutions may be stored and used almost indefinitely and that the seed culture, if it is a young actively growing culture at the time it is stored, may be used for a two-week period.

To determine the reproducibility of potency estimations obtained by this test, three tyrothricin solutions of varying concentration were assayed six times over a period of three weeks. Previously, such replicate assays had been made using the tyrothricin assay method described in the U. S. P. XIV (4). The results of these two series are shown in Table II.

It will be noted that when the U. S. P. test was used to assay the lowest potency solution, an average value of 78% was obtained, and that the individual tests ranged from 67 to 95%, a spread of 28%. When the modified test was used on a solution in this same range of concentration, an average of 77% was obtained with a spread of only 15%. For solutions assaying around 100 and 115% potency, the spread in the U. S. P. test was 34 to 38%, while in the modified test it is only 8 or 9%. This, we feel, indicates the improved reproducibility of the modified method, compared with the U. S. P. test.

A series of assays using both the modified and the U. S. P. method also was made of several tyrothricin-containing products. An extract of an ointment, a nose drop, and an eye wash were assayed four times by each method. The potency figures compared favorably, as is shown in Table III. It should be mentioned that because of the increased sensitivity to tyrothricin of the seed culture of the modified test, materials for assay may be diluted approximately ten times more than for the U. S. P. test, thereby reducing the possibility of interference with the assay by nontyrothricin ingredients in the products.

SUMMARY

The currently used Dubos-type tyrothricin assay has been modified by using a young seed culture and by using growth response rather than time to determine the test incubation period. Four levels of tyrothricin standard are used and a single concentration of each material to be assayed is included in the test. Both the standard dilutions and the inoculum are made in bulk and stored for use over a two-week period. The test has been designed so that a simple statistical formula can be applied to obtain 95 per cent confidence limits for each potency figure.

REFERENCES

(1) Dubos, R. J., *J. Exptl. Med.*, **70,** 1(1939).
(2) Dimick, K. P., *J. Biol. Chem.*, **149,** 387(1943).
(3) Reedy, R. J., and Wolfson, S. W., THIS JOURNAL, **39,** 1(1950).
(4) "United States Pharmacopeia," Fourteenth Revision, Mack Publishing Co., Easton, Pa., 1950, p. 650.
(5) Fisher, W. P., and Charney, J., to be published, 1951.
(6) Ciminera, J. L., to be published, 1951.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Eriodictin
Triethylenemelamine
1,2,3-Triaminopropane
sym.-Toluenetrisulfonic acid
Tetrahydrothiapyran
Propyl syringate
2-Sulfobenzoic acid
Sodium-*n*-hexadecyl sulfate
Sedoheptulose
ortho-Phthalamic acid
meta-Phenanthroline
3-Nitro-4-hydroxyphenylarsonic acid
Myricetin
Myoglobin
α-Methyl-δ-xyloside
3-Methylquinoline

A Study of the Polyethylene Glycols as Vehicles for Intramuscular and Subcutaneous Injection*

By CHARLES P. CARPENTER and C. BOYD SHAFFER

Tissue reactions at the site of subcutaneous and intramuscular injections of undiluted polyethylene glycol 300, propylene glycol, and peanut oil have been compared. The results obtained and described in this report indicate that a clinical trial of the polyethylene glycols as vehicles for certain drugs administered parenterally is justified.

THE POLYMERIC GLYCOLS (polyethylene, polyoxyethylene glycols) have enjoyed widespread use in the pharmaceutical industry as components of water-soluble ointment bases for topical application (1, 2). By reason of their unusual solubility characteristics, low order of toxicity, and wide range of compatibilities they have lent themselves to a diversity of uses. Such properties logically suggest the possibility of these compounds serving as carriers for drugs given by the subcutaneous or intramuscular routes, where water-solubility of the vehicle may be a highly desirable feature. Thus far, however, little has appeared in the literature regarding the suitability of the polyethylene glycols in such applications. Friedman (3) has proposed "Carbowax" compound 1500[1] as a vehicle for the intravenous administration of fat-soluble hormones, and has presented a limited amount of data on the tolerance of rabbits and dogs to doses of the polyglycol by this route.

The present report describes the results of animal experimentation designed to determine the tissue response to subcutaneous and intramuscular doses of the polyglycols, as well as the rate and extent of urinary excretion of these materials when injected in such fashion.

The polyethylene glycols are a series of moderately viscous, colorless, somewhat hygroscopic liquids with average molecular weights ranging from 200 to 600, and specific gravities of about 1.13. All are less volatile than glycerol. They dissolve in water in all proportions at room temperature to form transparent solutions, and are soluble in many organic solvents. They do not hydrolyze or deteriorate.

* Received September 22, 1951, from the Mellon Institute of Industrial Research, University of Pittsburgh, Pittsburgh 13, Pa.

[1] This product is a blend of substantially equal parts by weight of polyethylene glycol 300 and "Carbowax" compound 1540, the latter being a solid polyethylene glycol. The numbers refer to mean molecular weight. The name "Carbowax" is a registered trade mark of the Carbide and Carbon Chemicals Division of Union Carbide and Carbon Corporation.

EXPERIMENTAL

Samples.—Polyethylene glycols (PEG) 300 and 400 were used in this work. These were commercial products designated by the letter "W" signifying a water-white color. This designation has now been dropped by the manufacturer, since current production is entirely of the color-free grade.

Parenteral Injection.—To determine the response of tissue to subcutaneous or intramuscular administration of polyethylene glycol 300, the undiluted compound was injected into Sherman strain albino rats of 150 Gm. average weight. This was done with the animals under light sodium pentobarbital anesthesia in order to reduce the possibility of manipulative trauma. Subcutaneous injections were made in the abdominal region, while intramuscular injections were performed by inserting the needle at right angles into the multifidus muscle in the right lumbo-sacral region between the rib cage and thigh muscles. Groups of six rats were injected at each of the selected dosage levels and the animals were then dispatched in pairs at intervals of two, four or seven, and fourteen days. Gross observation of the area of injection was made, and in some few cases the affected part was taken for histopathological examination. Propylene glycol and peanut oil, materials currently in use as vehicles for parenteral administration, were used as controls on the PEG 300 under investigation.

Urinary Excretion of Polyethylene Glycols after Subcutaneous and Intramuscular Injection in Dogs.—The object of this phase of the investigation was to determine the extent of removal of the polyethylene glycols from subcutaneous and intramuscular depots. Four adult female mongrel dogs were used in this work. In performing an experiment, the animal was anesthetized with sodium pentobarbital given intravenously, and placed in the supine position upon a dog board. An indwelling catheter was placed in the bladder, and a constant intravenous infusion of saline at a rate of about 1 ml./min. was commenced via the jugular vein for the purpose of maintaining a satisfactory flow of urine. Urine was collected in volumetric flasks, and the bladder was washed with warm saline between successive periods of collection. Polyethylene glycol (2 ml./Kg.) was given subcutaneously in the thoracic region or intramuscularly in the hind legs. Because of the relatively large amount involved, the dose was divided among at least two and usually three to four sites.

With the animal in this position, urine was collected for successive time intervals up to six hours following injection of the dose. During this time it was occasionally necessary to supplement the anesthesia. At the end of six hours, the catheter and infusion tubing were removed, the animal was returned to a metabolism cage, and urine was col-

lected quantitatively up to forty-eight hours following the start of the experiment.

All urine samples were analyzed for polyethylene glycol by the gravimetric silicotungstate method (5). Factors for converting the weight of precipitate to polyglycol were determined by carrying out analyses upon standard solutions of the polyethylene glycols used in this study.

RESULTS AND DISCUSSION

The choice of dosage level was made on the assumption that for human intramuscular doses no more than 10 ml. of a therapeutic agent would be given in one locus. For a 50-Kg. person this would amount to 0.2 ml./Kg. By the application of a safety factor of 10, to cover the eventuality of greater human susceptibility, a 2 ml./Kg. dosage level for rats was derived. It was likewise anticipated that humans would receive a total dose no larger than 50 ml. by the subcutaneous route, and by the same reasoning this would correspond to a 10 ml./Kg. dosage level for rats if the safety factor of 10 was again employed. In some trials one-half and one-quarter of these levels were also administered to determine whether the volume of the dose had any appreciable effect upon the results.

Subcutaneous dosages of PEG 300 to rats at 10, 5, and 2.5 ml./Kg. produced a blanching of the overlying dermis within twenty-four hours after injection. At forty-eight hours this area was covered with a dry crust with underlying petechial hemorrhages. Increased vascularization and fibroblastic proliferation in the subcutaneous area were noted after four days. At the end of fourteen days, only slight vascularization remained. Propylene glycol produced tissue reactions that were indistinguishable from PEG 300. Peanut oil, on the other hand, was dispersed in globules in the subcutaneous tissue and caused no grossly visible reaction. No microscopic examination of the skin or underlying tissues was made.

Intramuscular injections to rats were made at dosage levels of 2 and 0.5 ml./Kg. Reflection of the skin from over the site of injection, forty-eight hours later, revealed vascular infiltration on the surface of the muscle. Examination of the transversely cut surface of the multifidus showed that the compound had been forced into muscle bundles and carried longitudinally producing ischemic necrosis of the individual fibers involved. This reaction was evident on gross and microscopic examination of rats killed on the second and seventh days after injection but not in those examined fourteen days after injection. When the dose displaced but did not penetrate muscle bundles, ischemic necrosis did not occur, but some increased vascularization and fibroblastic proliferation were seen. The latter response occurred consistently when the compound was injected parallel to the muscle bundles in the thigh as was demonstrated in several trials by this technique at equivalent dosage levels. There was slight swelling without inflammation, and no restriction of leg movement was detectable in rats so treated. The reactions produced by propylene glycol were again identical with those described for PEG 300. Peanut oil caused no reaction within the muscle and was found distributed in the form of walled-off globules of various sizes in nearby fat depots.

Histopathological examination of areas of ischemic necrosis, produced at the 2 ml./Kg. dosage level forty-eight hours after intramuscular injection, revealed displacement of cell nuclei and infiltration with inflammatory cells but with no evidence of any purulent exudate. The reaction may be characterized as a mild chemical inflammation. PEG 300 and propylene glycol produced responses which were indistinguishable in degree. Peanut oil apparently caused obliteration of the striations in the muscle but no inflammatory cells were found.

The LD_{50} for undiluted PEG 300 by the intravenous route for 90–120 Gm. female rats based upon a fourteen-day mortality period was 7.1 ml./Kg. This fact provides a considerable margin of safety in the event that the compound should be accidentally introduced directly into the blood stream. Detailed toxicological information on the polyethylene glycols has been published by Smyth, Carpenter, and Weil (4).

Doses of PEG 300 and PEG 400 do not elicit a foreign body reaction in the sense that they become encapsulated or walled off in subcutaneous or intramuscular depots. The experiments with dogs, using these routes of injection, demonstrate that removal of the polyglycol from such sites is rapid since the material diffuses freely into the surrounding tissues and vascular supply. The results of this work are summarized in Tables I and II, which show the extent of urinary excretion of the polyglycols after subcutaneous and intramuscular administration. High accuracy is not claimed for the determination at the twenty-four-hour and forty-eight-hour intervals since no attempt was made to remove interfering materials, other than sulfate, from these samples. The preceding samples were of much smaller volume

TABLE I.—URINARY EXCRETION OF POLYETHYLENE GLYCOL 300 FOLLOWING INJECTION OF 2 ML./KG. IN THE ANESTHETIZED DOG[a]

Dog No.	Wt., Kg.	Cumulative Excretion, % of Total Injected					
		1 Hr.	2 Hr.	4 Hr.	6 Hr.	24 Hr.	48 Hr.
				Subcutaneous			
517	22.1	0.9	5.1	15.7	25.7	76.2	96.4
510	11.3	3.5	13.9	32.3	47.1	91.2	93.3
510	11.3	2.9	9.9	27.4	41.5	92.7	95.1
				Intramuscular			
517	22.1	5.3	16.8	35.1	52.4	93.4	97.0
515	19.0	2.5	9.2	26.0	42.0	66.0	95.8
510	11.3	3.7	19.3	42.4	59.7	96.2	97.4

[a] Anesthesia discontinued after six hours.

TABLE II.—URINARY EXCRETION OF POLYETHYLENE GLYCOL 400 FOLLOWING INJECTION OF 2 ML./KG. IN THE ANESTHETIZED DOG[a]

Dog No.	Wt. Kg.	Cumulative Excretion, % of Total Injected 1 Hr.	2 Hr.	4 Hr.	6 Hr.	24 Hr.	48 Hr.
			Subcutaneous				
510	11.3	1.2	7.7	25.7	33.9	..	..
515	19.0	1.2	5.1	17.5	24.4	85.7	..
			Intramuscular				
516	15.7	4.3	11.4	27.6	34.7	..	..
517	21.0	4.5	11.9	26.8	24.0	87.4	90.3

[a] Anesthesia discontinued after six hours.

and were considerably more dilute as regards the normal urinary constituents, and corrections for interferences under these circumstances would be negligible. It is judged, therefore, that the over-all recovery at the end of two days is probably high. However, the data amply demonstrate that subcutaneously or intramuscularly injected doses of these polyglycols are removed from the site of injection with a fair degree of rapidity.

One consideration that might be raised by the clinician is the question of safety of the use of the polyglycols in individuals with impairment of kidney function. The renal excretion of the polyethylene glycols has been studied by Shaffer, *et al.* (5, 6), who have shown that PEG 400 is excreted by the dog at the level of glomerular filtration without tubular participation. Therefore, in the event of diminished renal activity, as long as any glomerular function exists there should be progressive removal of the material from the blood stream. Reference has been made above to the fact that the tolerance of experimental animals to PEG 300 by the intravenous route is high.

The intramuscular injections caused soreness and functional paralysis of the hindquarters of the dogs when the dosage of 2 ml./Kg. was equally divided and injected into the muscles of the hind legs. This functional paralysis lasted for several days and was followed by a period wherein the dogs moved slowly and stiffly. After fourteen to eighteen days all dogs were moving actively in normal fashion. When the dose was divided into four portions and administered in both the hind legs and shoulder muscles these reactions did not occur. All that was noticed was a transient stiffness which disappeared in about forty-eight hours. Both PEG 300 and 400 caused this same type of response. Doses of propylene glycol and peanut oil were not given to dogs.

SUMMARY AND CONCLUSIONS

Tissue reactions at the site of subcutaneous and intramuscular injections of undiluted Polyethylene glycol 300, propylene glycol, and peanut oil in rats have been compared.

Subcutaneous injection of PEG 300 or propylene glycol in dosages 2.5 to 10 times that anticipated for human use caused blanching of the skin and scab formation in forty-eight hours. After four days increased vascularization and fibroblastic repair tissue were present, but after fourteen days only slightly increased vascularization could be seen. Peanut oil dispersed in globules in the subcutaneous tissue and caused no visible reaction.

Intramuscular injection of 5 to 10 times the expected human dosage level of PEG 300 or propylene glycol produced ischemic necrosis of the muscle fibers when the dose infiltrated a muscle bundle. The tissue response may be characterized as a mild chemical inflammation. This reaction is a transient one as no evidence of injury was found fourteen days after the injection. Peanut oil caused no reaction within the muscle bundle, but accumulated in globules in nearby fat depots.

By the intravenous route undiluted PEG 300 has an LD_{50} for rats of 7.1 ml./Kg.

The major portion of subcutaneous or intramuscular doses of 2 ml./Kg. of undiluted PEG 300 or PEG 400 was excreted in the urine of dogs within twenty-four hours after injection.

It is concluded that the above findings justify the clinical trial of the polyethylene glycols as vehicles for the subcutaneous or intramuscular administration of drugs in such applications wherein their particular properties may be advantageous. Judged by the present results, these compounds are no more harmful under these circumstances than propylene glycol, on which there already exists a background of human experience.

REFERENCES

(1) Maynard, M. T. R., *J. Investigative Dermatol.*, **8**, 223 (1947).
(2) McClelland, C. P., and Bateman, R. L., THIS JOURNAL, *Prac. Pharm. Ed.*, **12**, 30(1949).
(3) Friedman, M. H. F., *J. Lab. Clin. Med.*, **29**, 530(1944).
(4) Smyth, H. F., Jr., Carpenter, C. P., and Weil, C. S., THIS JOURNAL, **39**, 349(1950).
(5) Shaffer, C. B., and Critchfield, F. H., *Anal. Chem.*, **19**, 32(1947).
(6) Shaffer, C. B., Critchfield, F. H., and Carpenter, C. P., *Am. J. Physiol.*, **152**, 93(1948).

The Evolution of the Concept of Isotonicity in Pharmacy*

By IVAN J. SZEKELY† and FRANK M. GOYAN‡

The concept of isotonicity in pharmacy arose at the turn of the century from earlier fundamental discoveries. Various approaches to the problem of determining quantities of substances required for the preparation of isotonic solutions that were adopted by pharmacists are discussed in the light of present knowledge. These considerations, some based upon the Arrhenius theory and others representing simple conversion of physical or biological methods, led to the gradual acceptance of the practice of adjusting solutions to conform to certain accepted isotonic values. The basis for choosing certain of these values is presented.

THE APPLICATION of physical chemistry to the problems of the physiologist prior to the 20th century is well summarized in a three-volume monograph by Hamburger (1). Very little theoretical work has been done in the last fifty years to modify the essential concepts of these early workers, but excellent summaries of experimental work relating the osmotic properties of pharmaceutical solutions to physical chemical measurements have been published (2, 2a, 3). Danielli (4) has taken the lead in establishing the difference between animal membranes and ideal semipermeable membranes, and Husa (5) has reported experiments showing that hemolysis of red blood cells is not prevented by certain substances at concentrations which might be calculated to be isotonic according to the physical chemical definition of isotonicity (6). Modern physiologists have not provided a method of approach to the problem of defining a term to replace isotonicity, and the medical practitioner seems content to accept as isotonic, solutions which are found to have the freezing point of blood serum (−0.53°, approximately). Substances like ammonium chloride, boric acid, urea, ethyl alcohol, glycerin, and others that have been reported to offer little or no protection against hemolysis may be regarded as exceptional, but there seems to be little tendency to provide different treatment of these substances in routine pharmaceutical practice.

Since the appearance of Hamburger's work (1), there has been very little fundamental change in the basis for calculating quantities required to produce isotonic solutions. In every case, the calculation is based upon the fundamental concept that the solution should be of such concentration as to exhibit freezing-point lowering or other colligative properties similar to blood serum. The Swiss Pharmacopœia, Fifth Edition, was the first pharmacopeia to define a method of calculating isotonicity based upon a value of −0.56° for the freezing point of blood serum. All present methods of calculation are based upon some value selected as representing the freezing point of blood serum, which, for healthy individuals, may range from a value of −0.51° to a value of −0.63° (3).

The freezing points of sodium chloride solutions for many different concentrations are well known from experimental work (7). A study of these values shows that a freezing point of −0.56° corresponds to a 0.95 per cent solution of sodium chloride, and that the presently accepted value of 0.90 per cent sodium chloride corresponds to a freezing-point lowering of −0.53°. Some pharmacopeias (8) specify lower concentrations of sodium chloride for normal saline solution. Fortunately the accuracy demanded in practice is not excessive, and solutions prepared according to any of these formulations serve satisfactorily. It is well known that for some time physicians requested that ophthalmic solutions be made equal in osmotic effect to a 1.4 per cent solution of sodium chloride. However, work done in 1930 (9) and extending up to the present time (10–12) indicates that lachrimal fluid is more nearly isotonic with blood serum (0.9 per cent NaCl). Custom, however, has established a 1.4 per cent NaCl value as an upper practical limit for the adjustment of ophthalmic solutions.

Because of the tolerance permitted in adjusting isotonic solutions, certain simplified assumptions have been introduced into the practical calculations. One of these is the assumption of additivity of the freezing-point contribution of various components of the solution. To be sure, this assumption is very nearly valid when the ionic strength of the finished mixture is similar to that of physiological salt solutions, but may deviate considerably for mixtures containing both non-

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electrolytes and electrolytes. It is known that the colligative properties upon which these calculations are based change considerably with concentration. Thus, it is essential that the additivity principle be applied only to properties measured for concentrations approximately in isotonic range.

Husa and Rossi (13) have summarized many of the early working rules for calculating the quantity of substances required to render a solution isotonic. The methods all depend upon some colligative property, the value of which is either estimated from chemical formula or determined experimentally. The great majority of the methods rely upon experimental determinations of freezing-point depression for some standard concentration, or some quantity easily derived from measured freezing-point depressions, such as the so-called sodium chloride equivalent which has been accepted as a measure of the osmotic contribution of various drugs (14–16). The sodium chloride equivalent is defined as that weight of the sodium chloride which will have the same osmotic effect as one similar weight unit of the substance in question. Sodium chloride equivalent values may be determined from molal freezing-point depressions selected at approximately isotonic concentrations (17) by setting up the algebraic statement that the actual freezing point of a solution of E grams of sodium chloride in any given volume is equal to the freezing point of a solution of 1 Gm. of the drug in the same volume. Both the 1 Gm. representing the weight of the drug and the E gram representing the weight of the sodium chloride must be divided by their corresponding molecular weights and multiplied by known values for the respective molal freezing-point depressions to obtain the two freezing-point values to be equated.

The principle underlying individual calculations based upon some previously agreed upon colligative property or its derivative is to calculate by simple proportion the osmotic contribution of each component to the finished solution. The various osmotic contributions, all reduced to a common denominator such as freezing-point depression or equivalent weight of sodium chloride, are added together and subtracted from the corresponding value for physiological salt solution or other standard. A variation of this technique of calculation is based upon computing the volume of water required to render all the added components isotonic with some accepted vehicle as a diluent (15, 16). Variations in the algebraic and graphical methods (17–20) have been introduced to facilitate presentation of this subject.

It may be assumed that all of the accepted methods for performing the numerical calculations based upon some standard osmotic property of the drugs are satisfactory. The choice of method is largely one of convenience in practice. However, serious errors can be made in assigning a value for the standard measure of osmotic effect, such as the sodium chloride equivalent. For drugs having known values for molal freezing-point depressions over a range of concentration sufficient to select a value corresponding to an isotonic concentration, the sodium chloride equivalent value is known with the same accuracy that may be assigned to the molal freezing-point depression, which is usually the order of magnitude of 1 per cent. However, a great many substances are encountered in practice for which adequate experimental determinations are not available. In many cases, these values may be approximated by assuming for the substance in question the average freezing-point value for its ionic type of strong electrolyte (17). There are, however, many substances that are not easily classified because some may be weak electrolytes or nonelectrolytes without the simple chemical formula giving sufficient evidence of these facts. Therefore, any serious attempt to refine the absolute precision of isotonic calculation must begin with an investigation of the colligative properties of various pure substances and mixtures at different temperatures (21).

REFERENCES

(1) Hamburger, H. J., "Osmotischer Druck und Ionenlehre," Vols. I–III, J. F. Bergman, Wiesbaden, 1902–1904.
(2) Lund, C. G., Peülicke Nielsen, E., and Pedersen-Bjergaard, K., "The Preparation of Solutions Iso-osmotic with Blood, Tears, and Tissue," William Heinemann, Ltd., London, 1947.
(2a) Lund, C. G., Pedersen-Bjergaard, K., Rasmussen, E. B., *Acta Pharm. Intern.*, **1**, 3(1950).
(3) Steiger, K., *Pharm. Acta Helv.*, **20**, 321(1945).
(4) Davson, H., and Danielli, J. F., "The Permeability of Natural Membranes," Macmillan Co., New York, 1943.
(5) Husa, W. J., and Adams, J. R., THIS JOURNAL, **33**, 329(1944).
(6) Scatchard, G., Hamer, W. J., and Wood, S. E., *J. Am. Chem. Soc.*, **60**, 3061(1938).
(7) "International Critical Tables," Vol. IV, McGraw-Hill Book Co., New York, 1928, p. 254.
(8) "Pharmacopée Française," 6th ed., L'Ordre National des Pharmaciens, Paris, 1937; "Pharmacopoea Hungarica," 4th ed., Opera Typographiae Reg. Hung. Publicae, Budapest, 1934; "United States Pharmacopeia," Eleventh Revision, Mack Publishing Company, Easton, Pa., 1936.
(9) Ridley, F., *Brit. J. Expil. Path.*, **11**, 217(1930).
(10) Krogh, A., Lund, C. G., and Pedersen-Bjergaard, K., *Acta Physiol. Scand.*, **10**, 88(1945).
(11) Hind, H. W., and Goyan, F. M., THIS JOURNAL, **38**, 477(1949).
(12) Revol, L., *et al.*, private communication.
(13) Husa, W. J., and Rossi, O. A., THIS JOURNAL, **31**, 270(1942).
(14) Goyan, F. M., "American Pharmacy," Vol. II, J. B. Lippincott Co., Philadelphia, 1947, p. 101.
(15) White, A. I., and Vincent, H. C., THIS JOURNAL, *Pract. Pharm. Ed.*, **8**, 406(1947).
(16) Sprowls, J. B., *ibid.*, **10**, 348(1949).
(17) Goyan, F. M., Enright, J. M., and Wells, J. M., THIS JOURNAL, **33**, 74(1944).
(18) Wells, J. M., THIS JOURNAL, *Pract. Pharm. Ed.*, **5**, 99 (1944).
(19) Nixon, W., and Culbert, R. C. A., *Pharm. J.*, **140**, 411(1938).
(20) Szekely, I. J., and Goyan, F. M., THIS JOURNAL, **41**, 32(1952).
(21) Huff, H., McBain, J. W., and Brady, A. P., *J. Phys. & Colloid Chem.*, **55**, 311(1951).

A Slide Rule for Isotonic Calculations*,†

By IVAN J. SZEKELY‡, FRANK M. GOYAN§, and HARRY W. HIND‡

The design and detailed instructions for the use of an isotonic calculator are presented. The calculator is a circular slide rule which is based upon the principle of the sodium chloride equivalent method for calculating isotonicity. The instrument is useful for rapid calculations in both the metric and apothecaries' systems at the prescription counter. It is designed to show 40 selected drugs, their sodium chloride equivalents, molecular weights, and a reference to the buffer range in which they are generally dispensed. A supplementary list of over 100 drugs with the above information is also provided.

THE numerous variations in isotonic calculations which appear in the literature (1) indicate dissatisfaction with conventional calculation routines, and express a need to simplify and improve them. During a postgraduate extension course for practicing pharmacists, one of the authors (I. J. S.) conceived the idea of creating a special slide rule that would perform the tedious elementary phases of isotonic calculations so as to allow more time for the discussion of the professional aspects of the subject. In executing this idea, the general problem of applying mechanical computing devices was approached broadly, several designs were tried and discarded, and finally it was decided to apply the principle of the circular slide rule to the method of calculation which is based upon the use of sodium chloride equivalent values.

The typical calculation combines multiplication, addition, and subtraction, a cumulative operation seemingly not suitable for conventional slide rules. However, by developing a series of semicircular scales, each corresponding to a different weight of drug, and each graduated uniformly in sodium chloride equivalent notation, it was possible to demonstrate that the sum of the sodium chloride equivalent contribution for each substance may be accumulated and read from a main scale. The operation involves the location of the sodium chloride equivalent value for each designated weight or fractional weight for each drug. Two radial cursors or markers are manipulated according to rules similar to those used in computing with standard circular slide rules. The markers transfer the information supplied for the various components of a prescription by the sodium chloride equivalent scales to an outer scale where the contribution of the various components are read as the total weight of sodium chloride to be added to the final prescription volume. An auxiliary scale is provided to show the volume of water required to render the combination of drugs isotonic with physiological salt solution, thereby placing the calculator at the disposal of those wishing to make use of the dilution methods (2, 3). The calculator has proved to be a great time saver, even for those who are thoroughly familiar with every phase of isotonic calculation. The entire calculation is performed for any combination of substances of known sodium chloride equivalent values without the aid of paper and pencil.

DESCRIPTION OF THE CALCULATOR

The most practical form of the calculator has been developed in two parts, one part being a mechanical system for causing sodium chloride equivalent values for different substances to appear in designated windows as required. This convenience is not to be confused with the essential arrangements of the scales as shown in Fig. 1. It will be noted from the figure that the scales are semicircular, the upper half of the circle being devoted to the metric system, and the lower half to the apothecaries' system thus providing two entirely independent calculators, one to be used for problems in the metric system and the other in the apothecaries' system. The construction of the scales is the same in principle for either system, and the detailed description of the metric scales given below also applies to the apothecaries' scales except for changes in units and values. It will be noted that the outer scale is divided uniformly so as to represent weights of sodium chloride up to 1.4 Gm. A corresponding uniform scale is divided so that 100 cc. coincides with 0.9 Gm. on the outer scale. These two scales are constructed in such a way that it is possible to read off the weight of sodium chloride in any volume of physiological salt solution. The outer scale is also used to translate the accumulated angle between two pointers into weight of sodium chloride corresponding to all of the substances considered. The two pointers are essentially two radial lines, each made on a separate strip of transparent plastic material which is fastened to a

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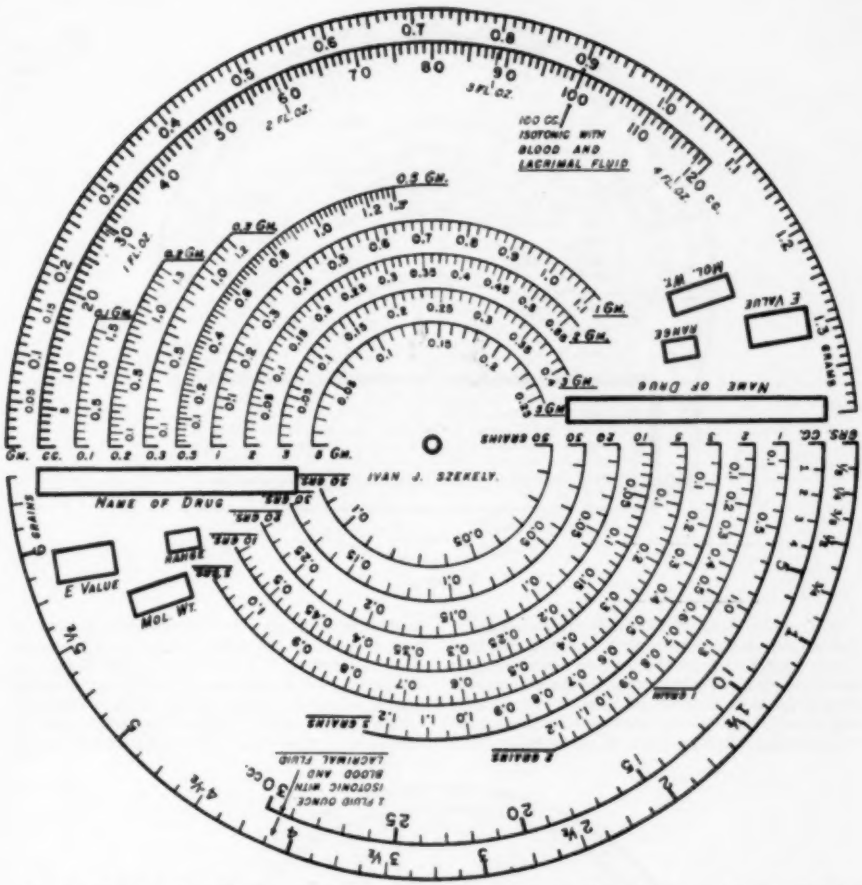


Fig. 1.—Photograph of the essential scales of the slide rule. Two radial pointers must be supplied (see text). The rectangular spaces, which appear near the origin of the E scales for both sections of the calculator, serve as guides in cutting windows used in experimental models to disclose E values and other data for 40 selected drugs printed in a matching pattern on a second disk. However, it is now believed that for special calculators not intended to be used with E values from extensive tables, the windows should be modified so as to replace the E scales entirely, or at least to take over their function for selected drugs by showing in appropriate spaces for each selected drug the corresponding numbers which otherwise would be read from each E scale.

center pivot contrived so that any angle established by the two pointers remains fixed while being swept over the scales. The operational variables are the angle between the pointers and the position of either pointer with respect to the scales.

TECHNIQUE OF CALCULATION

Figure 1 may be suitably mounted and supplied with two pointers in order to make a practical calculator to be used with sodium chloride equivalent values (E values) given in Table I. The pointers are easily constructed from sheet cellulose acetate or other suitable plastic material which permits engraving a thin straight line with ruler and sharp instrument. The engraved line may be blackened for easy reading, and should serve as a guide in drilling accurate center holes so that each pointer bears one centrally placed line extending from the center hole to the outer scale. The pivot may be a paper rivet or similar device which can be made to apply sufficient spring tension against a paper washer separating the pointers so as to stabilize the angle established by the operator.

Figures 2–5 are photographs showing the calculator in use. With the exception of the two outer scales, the other scales shown are sodium chloride equivalent scales (E scales), each scale representing a different weight unit of drug. These E scales are used with the pointers to perform the multiplication of the weight of drug under consideration times the

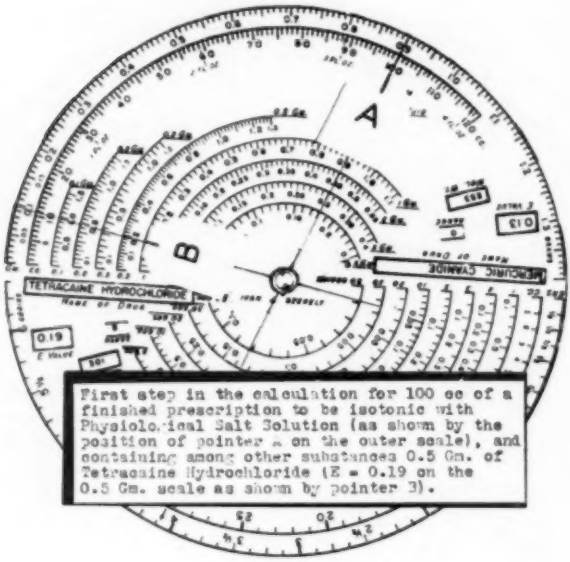

A
B
TETRACAINE HYDROCHLORIDE
First step in the calculation for 100 cc of a finished prescription to be isotonic with Physiological Salt Solution (as shown by the position of pointer A on the outer scale), and containing among other substances 0.5 Gm. of Tetracaine Hydrochloride (E = 0.19 on the 0.5 Gm. scale as shown by pointer B).

Figure 2

E value of the drug. Each such product may be read directly on the outer scale in terms of weight of sodium chloride. It will be noted from photographs that calculations are performed by choosing appropriate *E* scales for each integral weight or fractional weight needed to account for all of the substances in a prescription. Pointer A is set and held above the final weight of sodium chloride needed for the volume under consideration (normally 0.9 Gm. for 100 cc.). Pointer B is set above the first *E* value on the appropriate *E* scale corresponding to the weight of drug, thus establishing the first angle. Pointer B is then brought to zero while maintaining a constant angle, thus establishing a new position for pointer A

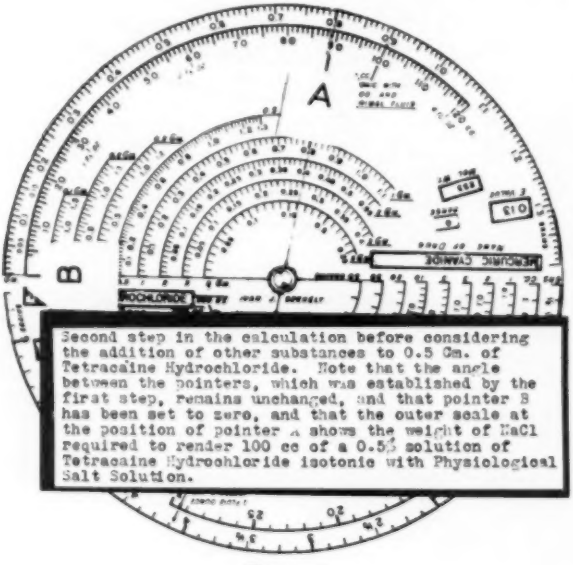
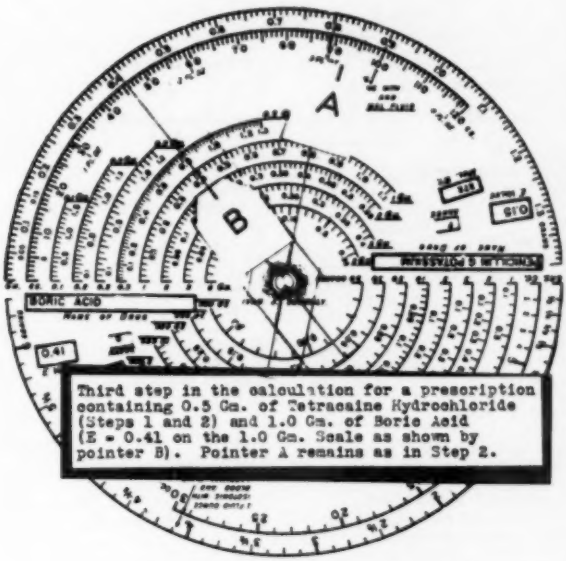

A
B
Second step in the calculation before considering the addition of other substances to 0.5 Gm. of Tetracaine Hydrochloride. Note that the angle between the pointers, which was established by the first step, remains unchanged, and that pointer B has been set to zero, and that the outer scale at the position of pointer A shows the weight of NaCl required to render 100 cc of a 0.5% solution of Tetracaine Hydrochloride isotonic with Physiological Salt Solution.

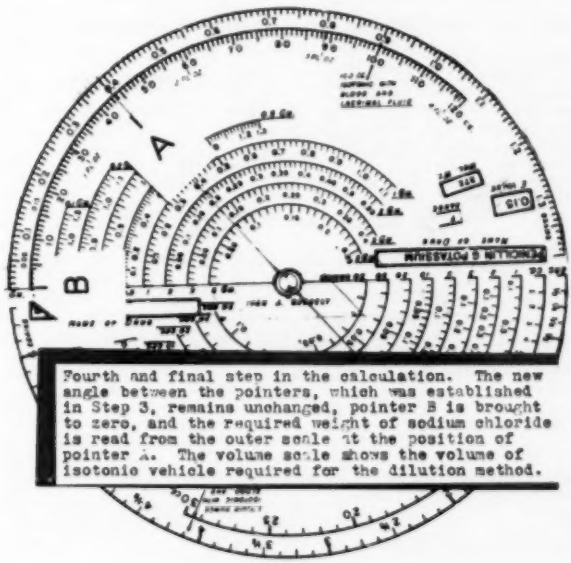
Figure 3



Third step in the calculation for a prescription containing 0.5 Gm. of Tetracaine Hydrochloride (Steps 1 and 2) and 1.0 Gm. of Boric Acid (E = 0.41 on the 1.0 Gm. Scale as shown by pointer B). Pointer A remains as in Step 2.

Figure 4

for further work and giving directly the weight of sodium chloride to be added if this were the terminal operation. This unit operation is repeated for a second drug, or the remaining fractional weight of the first, until all of the substances in the prescription have been accounted for. Each operation decreases the angle between the pointers. This angle is a measure of the weight of NaCl to be added (as read from the outer scale when pointer B is at zero). It may also be translated by direct reading from the volume scale into the required volume of diluent (isotonic with 0.9% NaCl), when using the dilution method which implies that sufficient distilled water is added to produce the final volume required.



Fourth and final step in the calculation. The new angle between the pointers, which was established in Step 3, remains unchanged, pointer B is brought to zero, and the required weight of sodium chloride is read from the outer scale at the position of pointer A. The volume scale shows the volume of isotonic vehicle required for the dilution method.

Figure 5

TABLE I

E Value	Medicinal Substance	Reference or Synonym	Mol. Wt.	Source Vehicle
1.08	Ammonium chloride	U. S. P.	53.50	(c)
0.13	Antipyrine	N. F.	188.22	(b)
0.19	Antistine hydrochloride	Antazoline hydrochloride N. N. R.	301.81	(a)
0.10	Atropine sulfate	U. S. P.	694.82	(a) II
0.11	Aureomycin hydrochloride	U. S. P.	544	(a)
0.20	Benadryl hydrochloride	Diphenhydramine hydrochloride U. S. P.	291.81	(a)
0.47	Boric acid	U. S. P.	61.84	(b)
0.10	Butacaine sulfate	U. S. P.	710.95	(a) I
0.10	Butyn sulfate	Butacaine sulfate	710.95	(a)
0.56	Calcium chloride ($2H_2O$)	U. S. P.	147.03	(c)
0.21	Camphor	U. S. P.	152.23	(a)
0.32	Carbachol	U. S. P.	182.65	(a) II*
0.10	Chloramphenicol	U. S. P.	323.14	(a)
0.18	Chloretone	Chlorobutanol	177.47	(a)
0.18	Chlorobutanol	U. S. P.	177.47	(a)
0.10	Chloromycetin	Chloramphenicol	323.14	(a)
0.14	Cocaine hydrochloride	U. S. P.	339.81	(b) I
0.13	Cupric sulfate ($5H_2O$)	U. S. P.	249.69	(b)
0.16	Dextrose (H_2O)	U. S. P.	198.17	(c)
0.15	Dibucaine hydrochloride	N. F.	379.92	(a) I
0.15	Dionin ($2H_2O$)	Ethylmorphine hydrochloride	385.88	(b) I
0.20	Diphenhydramine hydrochloride	U. S. P.	291.81	(a)
0.27	Disodium hydrogen phosphate ($7H_2O$)	Sodium phosphate	268.09	(c)
0.28	Ephedrine hydrochloride	U. S. P.	201.69	(b) II
0.17	Ephedrine sulfate	U. S. P.	428.54	(a) II
0.16	Epinephrine bitartrate	U. S. P.	333.29	(b) I
0.26	Epinephrine hydrochloride	.....	219.66	(a) I
0.14	Eserine salicylate	Physostigmine salicylate	413.46	(a) III
0.15	Ethylhydrocupreine hydrochloride	N. F. VIII	376.92	(a) I
0.15	Ethylmorphine hydrochloride ($2H_2O$)	U. S. P.	385.88	(b) I
0.18	Eucatropine hydrochloride	U. S. P.	327.84	(a) II
0.18	Euphthalmine hydrochloride	Eucatropine hydrochloride	327.84	(a)
0.27	Fluorescein sodium	U. S P.	376.27	(b) VI
0.34	Glycerin	U. S. P.	92.09	(c)
0.16	Holocaine hydrochloride	Phenacaine hydrochloride	352.85	(a)
0.16	Homatropine hydrobromide	U. S. P.	356.26	(b) II
0.11	Hyoscine hydrobromide ($3H_2O$)	Scopolamine hydrobromide	438.32	(b) II
0.15	Hyoscine hydrochloride ($2H_2O$)	Scopolamine hydrochloride	375.84	(a) II
0.09	Lactose (H_2O)	U. S. P.	360.31	(a)
0.30	Mecholyl chloride	Methacholine chloride	195.69	(a)
0.10	Merbromin	N. F.	750.70	(a)
0.12	Mercuric chloride	Mercury bichloride U. S. P.	271.52	(a)
0.13	Mercuric cyanide	.....	252.65	(a)
0.07	Mercuric oxycyanide	.....	469.24	(a)
0.10	Mercurochrome	Merbromin	750.70	(a)
0.12	Mercury bichloride	U. S. P.	271.52	(a)
0.30	Methacholine chloride	U. S. P.	195.69	(a)
0.19	Metycaine hydrochloride	Piperocaine hydrochloride	297.82	(a)
0.15	Morphine hydrochloride ($3H_2O$)	N. F.	375.84	(a)
0.10	Morphine sulfate ($5H_2O$)	U. S. P.	758.82	(a) II
0.23	Naphazoline hydrochloride	U. S. P.	246.73	(a)
0.19	Neostigmine bromide	U. S. P.	303.20	(a)
0.28	Neo-Synephrine hydrochloride	Phenylephrine hydrochloride	203.67	(a)
0.07	Nizin	Zinc sulfanilate	481.81	(a)
0.15	Nupercaine hydrochloride	Dibucaine hydrochloride	379.92	(a)
0.15	Optochin	Ethylhydrocupreine hydrochloride	376.92	(a)
0.25	Paredrin hydrobromide	Hydroxyamphetamine hydrobromide N. N. R.	232.13	(a)
0.16	Penicillin G sodium	U. S. P.	356.38	(a) V
0.16	Phenacaine hydrochloride (H_2O)	N. F.	352.85	(a) I
0.13	Phenazone	Antipyrine	188.22	(b)
0.32	Phenol	U. S. P.	94.11	(b)
0.28	Phenylephrine hydrochloride	U. S. P.	203.67	(a) II

(*Continued on page 37*)

TABLE I (*Continued from page 36*)

0.14	Physostigmine salicylate	U. S. P.	413.46	(*a*) III
0.11	Physostigmine sulfate	.....	648.45	(*a*) III
0.22	Pilocarpine hydrochloride	U. S. P.	244.72	(*b*) II
0.21	Pilocarpine nitrate	U. S. P.	271.27	(*a*) II
0.19	Piperocaine hydrochloride	U. S. P.	297.82	(*a*) I
0.19	Pontocaine hydrochloride	Tetracaine hydrochloride	300.82	(*a*)
0.40	Potassium acid phosphate	Potassium biphosphate	136.13	(*c*)
0.78	Potassium chloride	U. S. P.	74.55	(*c*)
0.24	Privine hydrochloride	Naphazoline hydrochloride	246.73	(*a*) II
0.18	Procaine hydrochloride	U. S. P.	272.77	(*b*)
0.19	Prostigmine bromide	Neostigmine bromide	303.20	(*a*)
0.20	Pyribenzamine hydrochloride	Tripelennamine hydrochloride	291.82	(*a*)
0.11	Scopolamine hydrobromide	U. S. P.	438.32	(*b*) II
0.32	Silver nitrate	U. S. P.	169.89	(*c*)
0.16	Silver protein (mild)	N. F.	.....	(*b*)
0.42	Sodium acid phosphate (H_2O)	Sodium biphosphate	138.01	(*a*)
0.72	Sodium bicarbonate	U. S. P.	84.02	(*c*)
0.42	Sodium biphosphate (H_2O)	U. S. P.	138.01	(*a*)
0.56	Sodium bisulfite	U. S. P.	104.07	(*a*)
0.35	Sodium borate ($10H_2O$)	U. S. P.	381.43	(*b*)
1.00	Sodium chloride	U. S. P.	58.45	(*c*)
0.41	Sodium iodide	U. S. P.	149.92	(*c*)
0.67	Sodium nitrate	.....	85.01	(*c*)
0.50	Sodium phosphate (anhyd.)	Na_2HPO_4 U. S. P.	141.98	(*c*)
0.40	Sodium phosphate ($2H_2O$)	$Na_2HPO_4 \cdot 2H_2O$	178.05	(*c*)
0.27	Sodium phosphate ($7H_2O$)	$Na_2HPO_4 \cdot 7H_2O$ U. S. P.	268.09	(*c*)
0.20	Sodium phosphate ($12H_2O$)	$Na_2HPO_4 \cdot 12H_2O$	358.21	(*c*)
0.60	Sodium propionate	N. F.	96.07	(*a*)
0.23	Sodium sulfate ($10H_2O$)	U. S. P.	322.22	(*a*)
0.57	Sodium sulfite (exsiccated)	N. F.	126.06	(*a*)
0.10	Sucrose	U. S. P.	342.30	(*c*)
0.16	Sulfacetamide	Sulamyd	214.24	(*a*) IV
0.16	Sulamyd	Sulfacetamide N. N. R.	214.24	(*a*) IV
0.21	Sulfadiazine sodium	U. S. P.	272.27	(*a*) IV
0.20	Sulfamerazine sodium	U. S. P.	286.29	(*a*) IV
0.20	Sulfanilamide	U. S. P.	172.21	(*a*) IV
0.19	Sulfathiazole sodium ($1^1/_2H_2O$)	U. S. P.	304.33	(*a*)
0.14	Syntropan	Amprotropine phosphate N. N. R. 1950	405.42	(*a*)
0.03	Tannic acid	N. F.	.....	(*b*)
0.19	Tetracaine hydrochloride	U. S. P.	300.82	(*a*) I
0.16	Trimeton maleate	Prophenpyridamine maleate N. N. R.	356.41	(*a*)
0.20	Tripelennamine hydrochloride	U. S. P.	291.82	(*a*)
0.53	Urea	U. S. P.	60.06	(*a*)
0.62	Zinc chloride	N. F.	136.29	(*c*) I
0.07	Zinc sulfanilate ($4H_2O$)	Nizin	481.81	(*a*) I
0.12	Zinc sulfate ($7H_2O$)	U. S. P.	287.56	(*c*) I

Explanation of Columns 5 and 6 of Table I

(*a*) Estimated from formula, Goyan, F. M., Enright, J. M., and Wells, J. M., THIS JOURNAL, **33,** 74 (1944).

(*b*) Calculated from Lund, C. G., Peülicke Nielsen, E., and Pedersen-Bjergaard, K., "The Preparation of Solutions Iso-osmotic with Blood, Tears, and Tissue," Heinemann, London, 1947.

(*c*) Calculated from "International Critical Tables," Vol. IV, McGraw-Hill Book Co., New York, 1928, p. 254.

I–V Solution numbers from Hind, H. W., and Goyan, F. M., THIS JOURNAL, **36,** 33(1947); Hind, H. W., Goyan, F. M., and Schwarz, T. W., *ibid.*, **36,** 413(1947).

VI Yalon, J. M., *Am. Profess. Pharmacist,* **16,** 360(1950).

I	Boric acid, C. P. (0.356 *M*)	22.0 Gm.
	Alkyldimethyl-benzylammonium chloride	1:50,000
	Distilled water, *q. s.*	1,000 cc.
II	Sodium acid phosphate (NaH_2PO_4) anhydrous	4.00 Gm.
	Disodium phosphate (Na_2HPO_4) anhydrous	4.73 Gm.
	Sodium chloride, C. P.	4.30 Gm.

(*Continued on page 38*)

TABLE I (*Continued from page 37*)

	Alkyldimethyl-benzylammonium chloride	1:50,000
	Distilled water, *q. s.*	1,000 cc.
II*	Physiological saline solution may also be used.	
III	Boric acid, C. P. (0.356 *M*)	22.0 Gm.
	Sodium sulfite (Na_2SO_3)	0.1%
	Phenylmercuric nitrate	1:100,000
	Distilled water, *q. s.*	1,000 cc.
IV	Distilled water is now believed to be the best vehicle for modern sulfa drugs.	
V	Sodium acid phosphate (NaH_2PO_4) anhydrous	0.56 Gm.
	Disodium phosphate (Na_2HPO_4) anhydrous	0.28 Gm.
	Sodium chloride	0.5 Gm.
	Distilled water, *q. s.*	100 cc.
VI	Boric acid, C. P.	2.00 Gm.
	Sodium carbonate (anhyd.)	0.82 Gm.
	Phenylmercuric nitrate (1:2,000)	4.0 cc.
	Distilled water *q. s.*	200.0 cc.

Figures 2-5 illustrate the calculation of the following prescription:

Tetracaine hydrochloride	0.5 Gm.
Boric acid	1.0 Gm.
Distilled water, *q. s. ad.*	100 cc.
Make isotonic	

The method described above is the most rapid, although the instrument is capable of being used in other ways. It will be noted that the volume scale is based entirely upon solutions isotonic with physiological saline solution, so that some of the simplifications resulting from the use of this scale are not available when working with solutions isotonic with a 1.4% solution of NaCl. However, alternate methods facilitate the use of the instrument when using a 1.4% solution of NaCl as the isotonic standard. Other features of the instrument include direct conversion of w/v per cent (Gm./100 cc.) to grains per fluid ounce merely by extending the two pointers so that they define a straight line reaching across a diameter.

It will be noted that on Fig. 1 several rectangles appear bearing as labels names of properties of drugs needed for calculations. In the construction of experimental models these rectangles are cut out to form windows which permit the appearance of pertinent information printed on a second rotating disk. This information is extended and presented in Table I. The table includes *E* values estimated or calculated from different sources, molecular weights introduced primarily for reference and identification, notations as to the source of the *E* value given, and suggested vehicles for many of the common ophthalmic drugs.

REFERENCES

(1) Szekely, I. J., and Goyan, F. M., THIS JOURNAL, **41**, 30(1952).
(2) White, A. I., and Vincent, H. C., THIS JOURNAL, *Pract. Pharm. Ed.*, **8**, 406(1947).
(3) Sprowls, J. B., *ibid.*, **10**, 348(1949).

☆

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Cyclobutanol
Cyclobutyl carbinol
p,p'-Di(dimethylamino)benzohydrol
α,α-Dichloroacetone
2,7,8,3',4'-Pentahydroxyflavan
5-Methyltryptophane
2-Methylthiazole
2-Methyl-8-sulfonic acid-1,4-quinone
1-Methyl 3,6-dihydroxycyclohexane
2-Hydroxy-6-methylterephthalic acid
Glycolamide
o-Fluorobenzoic acid
Ellagic acid
Dodecyl gallate
4,4'-Diphenyldiphenyl
1,7-Dimethylnaphthalene
1,1-Dimethylallyl mercaptan
1,6-Dimercaptohexane
Diiodoricinoleic acid
Dihydroconiferyl alcohol

The Effect of Roentgen Ray Irradiation on Rats Premedicated with Thyroxin and Thiouracil Derivatives*,†

By THOMAS J. HALEY, SAMUEL MANN, and ANDREW H. DOWDY‡

Hypothyroidism, induced by feeding nontoxic doses of thiouracil and its derivatives, does not significantly increase survival time nor decrease the total mortality of Roentgen ray irradiated rats. Propyl thiouracil significantly increased the rate of and total mortality of Roentgen ray irradiated rats. Hyperthyroidism produced by the subcutaneous injection of thyroxin into rats increased the rate of, and in one experiment, the total mortality of Roentgen ray irradiated rats. It was concluded that the potential sulfhydryl group in the thiouracil compounds is available for protecting the sulfhydryl groups in the animal organism, but a great excess must be present before any protectant effect can be observed. In such cases the drugs would be exerting pharmacological rather than antimetabolic effects.

HALEY, Mann, and Dowdy (1) pointed out that hyperthyroid rats were much more susceptible to the damaging effects of Roentgen ray irradiation than either normal or hypothyroid animals. In that investigation, the hypothyroid state was produced surgically to avoid the possible complication of sulfhydryl group protection from the potential sulfhydryl group in the molecules of the antithyroid drugs. During the past year, others have shown that, when extremely large doses of thiourea are injected into mice prior to irradiation, a significant decrease in mortality occurs (2, 3). Such effects may result by virtue of the mechanism of sulfhydryl group protection postulated by Patt, *et al.* (4), for the cysteine protection of Roentgen ray irradiated rats. However, the doses of thiourea employed, as well as the effective protectant time interval, indicate that the results obtained are due to a pharmacological effect of the drug and not to its antithyroid effect.

We would like to report a series of experiments in which the antithyroid effects of three thiouracil compounds were evaluated for their ability to modify Roentgen ray irradiation mortality in rats. To make the work comparable to our previous investigation, we included groups of hyperthyroid animals in each experiment.

EXPERIMENTAL[1]

Normal CFW strain male rats weighing 108–200 Gm. (average 154 Gm.) were maintained on Rockland rat diet and given 0.1% thiouracil, propyl thiouracil, or methyl thiouracil in their drinking water for periods varying from thirty-eight to fifty-four days. The variability of this time interval depended upon the rate at which the animals reached a weight plateau. All the animals were weighed weekly and when the weight plateau had been reached, the animals were separated into randomized groups of 20 animals each (see Table I). In each experiment selected groups of normal and hypothyroid animals were injected subcutaneously with 0.1 mg./animal/day of Squibb thyroxin for five days. It was evident that the thyroxin-treated animals were in a hyperthyroid state because they were more active and irritable than either the hypothyroid or normal animals. Furthermore, slight weight losses were observed in these thyroxin-medicated groups. Upon completion of the premedication period, the animals were subjected to 600 r acute whole body Roentgen ray irradiation. The technical factors were: 250 KVP; 15 MA; TSD 100 cm.; filters, 0.21 mm. Cu inherent, 0.5 mm. Cu parabolic and 1.0 mm. Al; HVL 1.85 mm. Cu; size of field–total body; r/minute measured in air 9.3 to 9.46. Uniformity of dosage was insured by rotating the radiation cage during treatment. The 250-KVP Picker Industrial Unit used was calibrated before each experiment with a Victoreen Thimble r meter. After irradiation the animals were returned to their regular cages and maintained on Rockland rat diet. The thiouracil compounds were not administered after irradiation. Autopsies were performed upon all animals dying during the thirty-day experimental period and upon all survivors at the end of that time. The usual signs of irradiation damage (diarrhea, bloody stools, anorexia, petechial hemorrhages, pale mucous membranes, etc.) were observed in all irradiated animals. Gross examination of the hypothyroid animals revealed thyroids which were blanched and slightly enlarged. Nonirradiated control groups of 20 animals each (normal, thyroxin-medicated, thiouracil-derivative-medicated, and thyroxin-thiouracil-medicated) were observed for thirty days.

RESULTS

The various nonirradiated control groups had no fatalities throughout the thirty-day experimental period. The results obtained with the other experi-

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‡ We wish to express our thanks to Dr. Stanton Hardy of Lederle Laboratories for the supply of thiouracil and propyl thiouracil.

[1] r = Roentgen; KVP = Kilovolt Peak; MA = Milliamperes; TSD = Target Subject Distance; and HVL = Half Value Layer.

mental groups are given in Table I where the LD_{50} days and slopes of the curves were calculated by the Litchfield method (5) using the formulas for cases where the upper portions of the curves are truncated. A set of typical time-per cent effect curves is shown in Fig. 1. Calculation of the slope ratios for the individual experiments and for the various types of treatments showed that the curves were essentially parallel in all cases. However, calculation of the reaction time ratios revealed that certain treatments had significant effects upon the mortality rate due to the Roentgen ray irradiation.

In the thiouracil experiment, there were no detectable differences in response between the control, thyroxin, thiouracil, and thyroxin-thiouracil groups indicating that the treatments employed had no real effect upon the rate of Roentgen ray mortality. There were, however, detectable differences in the responses of the thiouracil and thyroxin-thiouracil groups. These differences indicated that the animals receiving the thyroxin-thiouracil treatment were more susceptible to the lethal effects of Roentgen ray irradiation, as reflected in an increased rate of mortality.

In the propyl thiouracil experiment there were detectable differences in response which indicated that, at the dosage employed, propyl thiouracil increased the rate of mortality of the Roentgen ray irradiation animals. This increased rate of mortality is probably due to the irradiation and the antithyroid drug acting synergistically, because the control irradiated group had a lower rate of mortality and the antithyroid drug alone caused no fatalities during the experimental period. Detectable differences were also observed when the propyl thiouracil irradiated group response was compared with that of the thyroxin or thyroxin-propyl thiouracil groups. However, we were unable to detect any significant differences in response when the control irradiated, thyroxin, or thyroxin-propyl thiouracil groups were compared with each other.

In the methyl thiouracil experiment there were detectable differences between the responses given by the irradiated control and thyroxin groups which indicated that the latter treatment increased the rate of mortality after Roentgen ray irradiation. A similar relationship was also observed when the irradiated methyl thiouracil and thyroxin groups were compared. Such observations indicate that, in this particular experiment, hyperthyroidism was not conducive to survival after Roentgen ray irradiation. The other methods of treatment, thyroxin-methyl thiouracil, and methyl thiouracil alone, gave results which were not significantly different from those obtained with the irradiated control animals. This indicates that hypothyroidism induced by methyl thiouracil does not significantly increase survival time after Roentgen ray irradiation.

Inasmuch as the experiments herein reported were performed upon the same strain, age, sex, and average weight of animals, in quarters with the temperature maintained at 72 ± 2° F., during several seasons of the year, it was of interest to compare the reaction time ratios of the various similar groups to ascertain the effect of season upon the responses obtained. Season of the year had no significant effect upon the responses of the various irradiated control and thyroxin-antithyroid drug groups. Significant differences, which might be due to season, were observed in the response of the animals medicated with thyroxin in the winter. However, the data in Table I reveal that, in two of the three experiments performed, the thyroxin-medicated animals had an increased rate of mortality. This would indicate that hyperthyroidism increases the susceptibility of the animals to Roentgen ray irradia-

TABLE I.—INFLUENCE OF THIOURACIL COMPOUNDS AND THYROXIN ON IRRADIATION MORTALITY AND SURVIVAL TIME

Group	Actual % Mortality, 30 Days	Mortality Ratio	Median Survival Time (ST_{50}) in Days and Limits for Odds of 19/20	Slope Function and Limits for Odds of 19/20	Season of Year
Control	60	12/20	15 (12.5 to 21.0)	1.46 (1.24 to 1.72)	Summer, 1950
Thiouracil	60	12/20	19 (14.4 to 25.7)	1.83 (1.40 to 2.38)	
Thyroxin thiouracil	70	14/20	12.5 (10.3 to 15.1)	1.52 (1.29 to 1.79)	
Thyroxin	60	12/20	12.3 (8.8 to 17.2)	2.0 (1.47 to 2.70)	
Control	60	12/20	18.6 (13.8 to 25.1)	1.84 (1.41 to 2.39)	Fall, 1949
Propyl thiouracil	80	16/20	10.6 (8.5 to 13.3)	1.67 (1.4 to 2.0)	
Thyroxin propyl thiouracil	50	10/20	20 (13.4 to 29.8)	2.12 (1.42 to 3.16)	
Thyroxin	55	11/20	18.6 (13.7 to 25.3)	1.84 (1.4 to 2.43)	
Control	60	12/20	16.2 (13.2 to 19.8)	1.55 (1.28 to 1.88)	Winter, 1951
Methyl thiouracil	50	10/20	15.0 (11.9 to 18.9)	1.55 (1.25 to 1.92	
Thyroxin methyl thiouracil	50	10/20	12.5 (8.9 to 17.5)	1.87 (1.33 to 2.62)	
Thyroxin	85	17/20	10.0 (8.4 to 11.9)	1.48 (1.28 to 1.70)	

tion damage. Comparison of the reaction time ratios of the antithyroid drug series shows that, whereas thiouracil and its methyl derivative had no real effect upon the mortality rate of the irradiated animals, propyl thiouracil significantly increased both the rate of and the total mortality. This points to a direct toxic effect of the drug rather than a seasonal variation in animal response, because the thyroxin-propyl thiouracil group, in which the effect of the antithyroid drug was counteracted, gave a response similar to the irradiated control group.

DISCUSSION

Upon the basis of the results herein reported as well as those obtained earlier (6–8), it is quite evident that the hypothyroid state, surgically or chemically induced, does not protect animals from the lethal effects of Roentgen ray irradiation. It is also evident that hyperthyroidism is not conducive to survival after such irradiation.

In so far as sulfhydryl compound protection of irradiated animals is concerned, there is ample evidence that the prior injection of such compounds does protect the animal from irradiation lethality (4). Furthermore, the prior injection of thiourea, an antithyroid drug, also protects the animals (2, 3). However, in all instances, the amount of drug administered was within the toxic range. When thiourea is fed to the animals in a nontoxic dose, no protectant effect can be demonstrated even though the animals are definitely hypothyroid (8). Our present results indicate that thiouracil and its derivatives also have no protectant effect under similar circumstances. It would appear, therefore, that the sulfhydryl-containing compound must be present in the animal body in a great excess to exert any protectant effect against Roentgen ray irradiation damage. Furthermore, it is also evident that the potential sulfhydryl group in the molecule of thiouracil and its derivatives is available for such protectant action. However, sulfhydryl compounds are of no practical use in irradiation protection of humans although they are extremely useful as laboratory tests for elucidating part of the mechanism whereby ionizing radiations produce damage to the organism.

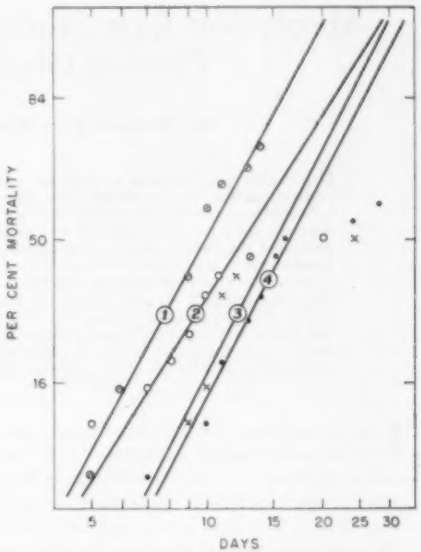

Fig. 1.—Radiation mortality and metabolism. ① Thyroxin. ② Methyl thiouracil thyroxin. ③ Methyl thiouracil. ④ Control.

REFERENCES

(1) Haley, T. J., Mann, S., and Dowdy, A. H., *Endocrinology*, **48**, 365(1951).
(2) Limperos, G., and Mosher, W. A., *Science*, **112**, 86 (1950).
(3) Mole, R. H., Philpot, J. St. L., and Hodges, G. R. V., *Nature*, **166**, 515(1950).
(4) Patt, H. M., Tyree, E. B., Straube, R. L., and Smith, D. E., *Science*, **110**, 213(1949).
(5) Litchfield, J. T., Jr., *J. Pharmacol. Exptl. Therap.*, **97**, 399(1949).
(6) Blount, H. C., Jr., and Smith, W. W., *Science*, **109**, 83 (1949).
(7) Haley, T. J., Mann, S., and Dowdy, A. H., *ibid.*, **111**, 333(1950).
(8) Haley, T. J., Mann, S., and Dowdy, A. H., *ibid.*, **114**, 41(1951).

Absorption Rate Studies of Orally Administered Cardiac Glycosides in Cats*,†

By WALLACE F. WHITE and OLE GISVOLD

Digitoxin, acetyl digoxin, digoxin, and lanatoside C were administered orally to unanesthetized cats in single fatal doses. Physical state of subdivision and lipoid solubility appeared to modify rate of absorption. Digitoxin, acetyl digoxin, and digoxin were rather uniformly and nearly completely absorbed within a few hours, provided they were dissolved in aqueous alcohol or aqueous "Tween 80" solutions. Lanatoside C was slowly absorbed in alcoholic solution. Digoxin and lanatoside C tablets were absorbed very slowly. Lanatoside C is much less lipoid-soluble than the others. It is postulated that high lipoid solubility of digitoxin and acetyl digoxin assures absorption of solid and dissolved states, whereas low lipoid solubility retards absorption of digoxin tablets and lanatoside C in solid or in solution. Acetyl digoxin deserves clinical trial as a substitute for digitoxin. Digoxin in alcohol or "Tween 80" solution should be as potent as digitoxin in solution.

THE crude plant mixtures of *Digitalis purpurea* and certain other species have been used in medicine by oral administration for a period of time long enough to acquaint the medical profession with their virtues and limitations. The recent trend toward the use of purified cardiac glycosides is evidence that limitations have been prominent. At present much attention is being given to the oral use of digitoxin. Some reactions have been unfavorable, however, since many cases of toxicity have been reported (1). Of the many additional cardiac glycosides which have been isolated in relatively pure form, two have received considerable clinical trial, namely digoxin and lanatoside C.

The information which is available concerning intestinal absorption of these three clinically used cardiac glycosides has been of a sketchy nature for the most part. Digitoxin is stated to be completely absorbed when given orally (2). In a recent report (3) lanatoside C has been stated to be 19 per cent absorbed in cats after oral administration, which corresponds with the dosage differential between digitoxin and lanatoside C given in U. S. P. XIV. Batterman and DeGraff (4) were able to digitalize patients with oral doses of digoxin ranging between 0.5 and 1.0 mg. given every six hours. The average U. S. P. XIV oral doses for these drugs are 0.1 mg. for digitoxin, 0.5 mg. for digoxin, and 0.5 mg. for lanatoside C, all in tablet form.

In the present study it was intended to compare intestinal absorption of these three commonly used cardiac glycosides in cats under nearly identical experimental conditions. We then planned to evaluate by comparison the absorption of a recently isolated cardiac glycoside, acetyl digoxin, which has been made available in quantity by the researches of Gisvold and Hopponen (5) and Gisvold and Krishnamurty (6). Acetyl digoxin is obtained from leaves of *Digitalis lanata* and may be isolated with greater ease than the methods employed in the extraction of digitoxin from *Digitalis purpurea* or of lanatoside C from *Digitalis lanata*. If absorption is shown to be satisfactory in an experimental animal, this drug may be made available for clinical study as a new therapeutic agent to compete with digitoxin, digoxin, and lanatoside C.

The comparison of the drugs chosen for study has further interest because of the chemical similarity between the four glycosides. The differences in structure between the other three glycosides and digoxin are as follows: the absence of the hydroxyl group at C_{12} in digitoxin, the addition of an acetyl group in acetyl digoxin and an acetyl group plus glucose in lanatoside C. Such differences are known to cause marked changes in solubility of compounds of this type. Another purpose for this study, therefore, has been to attempt to find a correlation between solubility and rate of absorption of cardiac glycosides from the intestinal tract.

METHOD

Rate of absorption can be studied in two ways, first by determining the quantities of various concentrations of a given substance absorbed in unit time, and secondly by determining the varying periods of time required to absorb a definite quantity of substance when administered in different concentrations (7). The latter method has been employed in this study in which death was assumed to result when a definite fatal amount of drug had been absorbed into the blood stream and subsequently distributed to the heart muscle.

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Presented to the Scientific Section, A. PH. A., Buffalo meeting, August, 1951.
† This investigation was supported by research funds of the Graduate School of the University of Minnesota.

It was considered worth while to study rate of absorption of the molecularly dispersed state as well as the solid form of each drug. Three forms of solutions were used to administer the drugs in a dispersed state, namely aqueous alcoholic solution, aqueous "Tween 80"[1] solution, and a combination of the two having a lower alcohol content. The drugs were supplied in highly purified form intended for experimental use.[2]

The solid form of the drug consisted of Crystodigin tablets,[3] Cedilanid tablets,[4] digoxin tablets,[5] and acetyl digoxin powder prepared in our laboratory and placed in capsules after being diluted with lactose.

Cats were chosen as experimental animals because a large amount of data are available concerning intravenous lethal doses of cardiac glycosides in this animal. This information provides a basis for determining the quantity of drug absorbed, since death was the end point used to indicate the time required for absorption of an amount of drug comparable to that required to kill by intravenous administration. Unanesthetized cats which had fasted overnight were placed on animal boards for varying periods of time, usually seven hours when they survived that long.

Quantities of drugs between 10 and 30 mg. were dissolved in alcohol and diluted to 25 ml. of 50% aqueous alcohol stock solutions and placed in rubber-stoppered vials. Withdrawals were made with a fixed volume standardized syringe which delivered 0.974 ml. of liquid at room temperature. The proper dose consisting of one or more syringefuls of stock solution diluted to 10 ml. with 20% alcohol or water in the "Tween 80" experiments was administered by stomach tube. This was followed by 20–30 ml. of water to assure administration of the full dose. Tablets or individually weighed capsules of acetyl digoxin were placed in the mouth and swallowing induced by 5-ml. portions of water until a total of about 20 ml. of water was given.

The survival time after the single oral dose was determined in minutes, plus or minus five minutes in most cases. Animals which were alive at the end of the day were placed in cages and were unobserved thereafter. Some died during the night. Those cats which lived beyond six days were arbitrarily considered to have recovered from acute toxicity. These recovered animals were titrated intravenously to death at various intervals of time after the sixth day with digitalis tincture in order to indicate whether there was evidence of permanent damage due to the earlier oral drug treatment.

RESULTS

Since vomiting did not occur, except in one case, during the periods of observation while the cats were immobilized, it is apparent that under these circumstances vomiting due to cardiac glycosides in relatively pure form is not a local phenomenon. This absence of vomiting was fortunate since full absorption was not hindered. Vomiting was seen on many occasions after the first day in those animals which survived the initial part of the experiment, therefore one could not be certain that all of the drug had remained in the intestinal tract long enough for complete absorption in those animals surviving for several days.

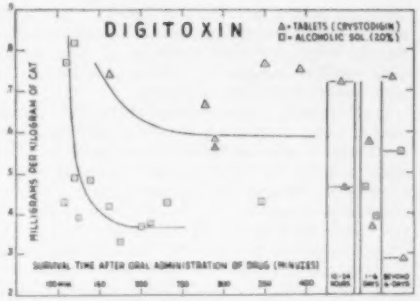
DIGITOXIN
Δ = TABLETS (CRYSTODIGIN)
□ = ALCOHOLIC SOL. (20%)
MILLIGRAMS PER KILOGRAM OF CAT
SURVIVAL TIME AFTER ORAL ADMINISTRATION OF DRUG (MINUTES)

Fig. 1.—Unanesthetized cats were given single oral doses of digitoxin in 10 ml. of 20% alcohol, followed by 30 ml. of water by stomach tube. The tablets were given by mouth followed by 20–30 ml. of water in 5-ml. portions to induce swallowing.

When the highest doses of lanatoside C were used, most of the animals defecated rather loose stools during the six- to eight-hour immobilization periods. These doses were 10 to 14 times the average intravenous lethal dose for this drug (8). Thus in the high concentrations used, the drug, appeared to produce a local irritating action on the intestinal tract to induce defecation, rather than irritating action on the stomach to induce vomiting.

The experimental results are presented in graph form in Figs. 1–4. It will be noted that great contrast exists between absorption rates of solution and solid form of all the drugs with the exception of lanatoside C. Let us first consider the data referring to the rates of absorption of the dispersed states of

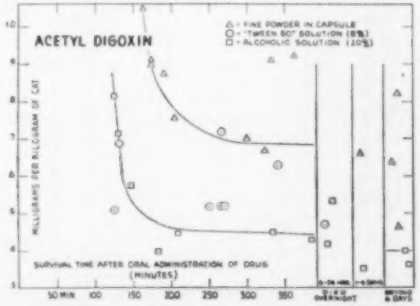
ACETYL DIGOXIN
Δ = FINE POWDER IN CAPSULE
○ = "TWEEN 80" SOLUTION (8%)
□ = ALCOHOLIC SOLUTION (20%)
MILLIGRAMS PER KILOGRAM OF CAT
SURVIVAL TIME AFTER ORAL ADMINISTRATION OF DRUG (MINUTES)

Fig. 2.—Unanesthetized cats were given single oral doses of acetyl digoxin in 10 ml. of 20% alcohol or 8% "Tween 80" in water by stomach tube, followed by 30 ml. of water. Capsules containing finely ground powder were placed in the mouth and washed down with 20–30 ml. of water in 5-ml. portions.

[1] We are indebted to the Atlas Powder Company, Wilmington, Del., for the nonionic emulsifier known as "Tween 80."
[2] We are indebted to Winthrop-Stearns, Inc., New York, for the digitoxin; to the Sandoz Chemical Works, Inc., New York, for the lanatoside C, and Burroughs Wellcome & Co., Inc., Tuckahoe, N. Y., for the digoxin.
[3] A brand of digitoxin manufactured by Eli Lilly & Co., Indianapolis, Ind.
[4] A brand of lanatoside C supplied by Sandoz Chemical Works, New York.
[5] Supplied by Burroughs Wellcome & Co., Inc., Tuckahoe, N. Y.

digitoxin, acetyl digoxin, and digoxin. The minimum time required to kill regardless of concentration was approximately two hours in all three drugs. This would be expected if the drugs are assumed to have qualitatively similar actions.

Such uniformity is nonexistent at the other asymptote of the curves which considers minimum concentration of drug needed to kill the animals. This latter part of the curve appears to reflect rate of absorption, whereas the minimum time axis is independent of concentration. It will be seen that the smoothed curve levels off at about 105% of the intravenous dose in the case of digitoxin considering 0.33 mg./Kg. of cat as the average intravenous lethal dose (8). The corresponding estimates for the other glycosides are 115% for acetyl digoxin with an average intravenous lethal dose in six cats of 0.39 mg./Kg. (9), and 160% for digoxin which has an average intravenous lethal dose of 0.21 mg./Kg. of cat (8). The lanatoside C estimate cannot be obtained from our data, however, the average intravenous lethal dose in cats is 0.25 mg./Kg. (8). Figure 4 in contrast with the other figures emphasizes the poor absorption of lanatoside C in solution. These estimates are rough approximations intended only to point out differences between the glycosides studied.

The reason for using "Tween 80" in some of the experiments was to attempt to rule out possible effects due to alcohol as a vehicle. The results indicate that "Tween 80" is comparable to alcohol for retaining the cardiac glycosides in the molecularly dispersed state.

The greater lag in fatal action observed in digitoxin and acetyl digoxin in solid form compared with the same drugs in solution would seem to be accounted for by the additional time required to solubilize the solids in the intestinal tract, if it is assumed that substances must be in solution to be absorbed (7). The very slow action of digoxin and lanatoside C in tablet form might indicate considerable difficulty in becoming dissolved in the intestinal secretions.

All four glycosides administered in solid form indicated minimal concentrations needed to kill at higher levels than in solution (Figs. 1–4). Within the seven-hour observation period only about half of the digitoxin and acetyl digoxin each was absorbed. The other drugs were very much slower and presumably much less completely absorbed.

The animals which demonstrated such objective signs of recovery as eating regularly, absence of vomiting, and normal alert attitude in the cages were titrated intravenously with a sample of tincture of digitalis made from University of Minnesota grown *Digitalis purpurea* leaves which had been tested on six untreated cats. This tincture had been compared with U. S. P. reference standard tincture of digitalis also tested on six untreated cats. Table I compares the intravenous lethal doses of nine experimental animals previously given the oral drug treatment with the 12 controls.

In Table II are listed distribution coefficients obtained when 50 ml. of chloroform was shaken with 60 ml. of 16% aqueous methanol solution containing 100 mg. of glycoside. Methanol was required to dissolve the glycosides.

DISCUSSION

One might question whether vomiting due to local action should be delayed for such a long period of time as suggested by Gold, *et al.* (3). They

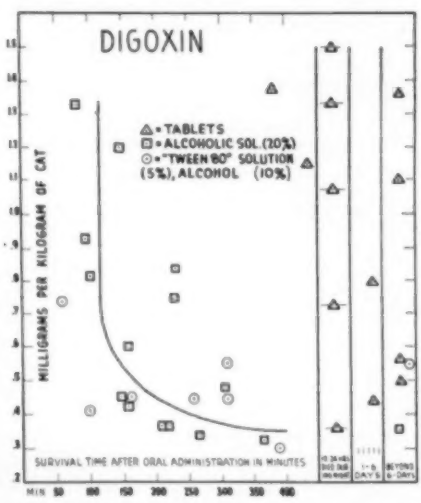
DIGOXIN
△ = TABLETS
□ = ALCOHOLIC SOL (20%)
⊙ = "TWEEN 80" SOLUTION (5%), ALCOHOL (10%)
MILLIGRAMS PER KILOGRAM OF CAT
SURVIVAL TIME AFTER ORAL ADMINISTRATION IN MINUTES
1-5 DAYS
BEYOND 5 DAYS

Fig. 3.—Unanesthetized cats were given single oral doses of digoxin in 10 ml. of 20% alcohol or in aqueous "Tween 80" solution, followed by 30 ml. of water by stomach tube. Tablets were placed in the mouth and washed down with 20–30 ml. of water in 5-ml. portions.

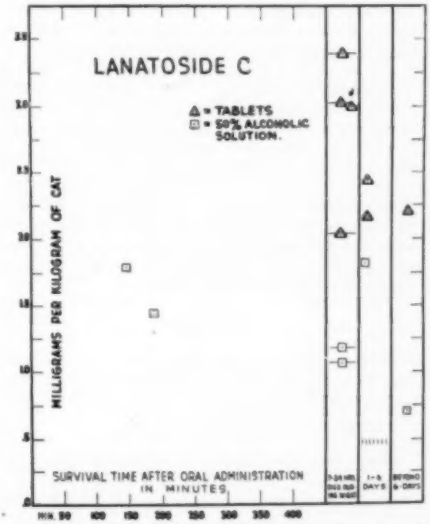

Fig. 4.—Unanesthetized cats were given single oral doses of lanatoside C in 10 ml. of 50% alcohol, followed by 30 ml. of water. Tablets were placed in the mouth and washed down with 20–30 ml. of water in 5-ml. portions.

TABLE I

Cat Wt., Kg.	Oral Dose, Mg./Kg.	I. V. Lethal Dose, Mg./Kg. Std.	U. of M.	Difference Between Oral and I. V. Tests Days	Weight, %
2.6	(Controls)	75			
2.3		69			
1.7		64			
2.8		79			
2.3		94			
2.0		75			
		76 (av.)			
1.9	(Controls)		54		
2.4			66		
2.4			81		
2.7			51		
2.4			52		
2.2			69		
			62 (av.)		
1.8	0.37 (acetyl digoxin 20% alc.)		16	5	−16
4.1	0.40 (acetyl digoxin 20% alc.)		20	7	−12
1.9	0.40 (digoxin tablets)		57	10	−16
2.2	0.46 (digoxin tablets)		24	10	− 5
2.3	1.10 (digoxin tablets)		66	38	+ 9
1.9	0.55 (digitoxin 20% alcohol)		11	13	− 9
2.1	0.29 (digitoxin tablets)		51	19	+ 5
2.5	0.73 (digitoxin tablets)		78	30	−12
1.8	0.45 (lanatoside C 50% alc.)		44	41	− 5

studied four poorly absorbed glycosides, one of which was lanatoside C by oral administration in cats. The average time to vomiting for lanatoside C was slightly under seven hours in their report. The highest dose used by them was 0.8 mg./Kg. With one exception all of our doses of lanatoside C were above 1.0 mg./Kg., the highest dose being 3.4 mg./Kg. Of 14 cats given lanatoside C orally only one vomited. This cat had received 1.4 mg./Kg. in 50% alcoholic solution by stomach tube and vomited within ninety minutes. All others failed to vomit during the seven or more hours during which time they were fastened to the animal boards. Those that survived vomited on the next day. One cat given 2.0 mg./Kg. in tablets lived ten days and vomited and retched intermittently during most of that period of time. None of the 98 cats tested with the other drugs vomited during the immobilization period and only a few defecated.

Time-concentration reactions are discussed in considerable detail by Clark (11). In reference to delayed action he used as an example hyperbolic curves obtained by Straub when digitoxin and ouabain were plotted, time of systolic arrest against concentration. Delay of about five minutes was seen when digitoxin was used and about thirty minutes when ouabain was used on isolated frog hearts. He also remarked that a minimum concentration undoubtedly exists below which an action could not be produced in infinite time.

Our findings with intact cats are similar with respect to the type of curves obtained but different in so far as latent period is concerned. The latent periods were about two hours when various concentrations of molecularly dispersed states of digitoxin, acetyl digoxin, and digoxin were plotted against survival time in cats. Our data suggest similar qualitative actions for the three glycosides. The concentrations of lanatoside C, though much higher than the other glycosides studied, were not sufficiently high to indicate a limiting time required to cause death.

Johannes Ipsen (12) studied time-concentration reactions in mice determining time of death after injection of various concentrations of bacterial toxins. According to him the general formula which describes fatal time-concentration reactions in animals is as follows:

$$(D/d - 1)^a(T/t - 1) = K$$

in which a and K are constants to be determined for each toxin, D/d equals dose over minimal dose, and T/t equals mean survival time over minimal survival time.

TABLE II

Drug	Distribution Coefficient (Chloroform), Mg.
Digitoxin	96.5
Acetyl digoxin	98.0
Digoxin	81.5
Lanatoside C (10)	16.2

Our data are not extensive enough to determine constants, but are sufficient to point out the similarity between the time-action curves obtained in mice treated with bacterial toxins and cats treated orally with cardiac glycosides. With more data it should be possible to use this same general formula and calculate the constants for any given cardiac glycoside. This should provide the basis for a biological assay method in which intestinal absorption would be taken into account. Objections have been raised to the use of intravenous injections in order to determine the potency of drugs which are normally used by oral administration (13).

The drugs given in solid form had to be dissolved before absorption could take place. This added latent period beyond that obtained in solution is seen in Figs. 1 and 2 in which two curves appear; one for undissolved drug and one for solution. All four of

the drugs studied are classed as water-insoluble compounds, but that doesn't imply that they are completely insoluble in water. Since they are very potent substances even slight solubility may be sufficient in the concentrations required for action.

Another factor in addition to water solubility which may play an important role is lipoid solubility. Höber (7) calls attention to this fact in referring to intestinal absorption by stating that lipoid solubility plays the same role in tissue permeability as in cell permeability. He states further that substances which are practically lipoid-insoluble, as well as of nearly the same molecular volume, can differ enough in lipoid solubility to cause definite differences in the rate of absorption. The lipoid solubility of lanatoside C is presumed to be much lower than the other compounds tested according to Table II since the chloroform aqueous-methanol partition coefficient is much lower for lanatoside C. In addition, it is soluble in water only to the extent of 1 part in 20,000 according to U. S. P. XIII. Figure 4 illustrates the poor absorption of this drug both in solution and in solid form.

Digoxin is intermediate between lanatoside C and the others in lipoid solubility according to Table II. Digoxin tablets are slowly absorbed but solutions are rapidly absorbed (Fig. 3). Only in digitoxin and acetyl digoxin with very high lipoid solubility do we find rapid absorption of solid and solution forms of the drugs (Figs. 1 and 2).

In reference to therapeutic value one might question the choice of digoxin or lanatoside C tablets. On the other hand if digoxin were administered in solution orally, it would be necessary to reduce the dose markedly in order to avoid toxicity. Digoxin dissolved in alcohol and added to "Tween 80" and placed in capsules after removal of part or all of the alcohol should provide a potent and quickly absorbed cardiac glycoside preparation. A dose of 0.1 mg. of digoxin in solution should cause action comparable to 0.1 mg. digitoxin in tablet form and perform its action more promptly. It is necessary to assume that a slow rate of absorption means incomplete absorption. Evidence for this lies in the fact that in tablet form the average maintenance doses for digoxin and lanatoside C have been 0.5 mg., in comparison with 0.1 mg. for digitoxin.

Acetyl digoxin has not been reported to have been used clinically. In view of its good absorption after oral administration in cats it should compare favorably with digitoxin if the difference in potency, 85% as potent as digitoxin, is taken into consideration. It deserves to be given clinical trial to test other qualities such as accumulation.

Digitoxin in solution has not been popular for oral use because of the danger of having the patient take an incorrect dose after careless manipulation of a dropper or other measuring device. This objection could be overcome by the use of capsules as suggested for digoxin. The capsules would be more quickly and possibly more completely absorbed than tablets. Complete digitalization should be accomplished in a few hours by oral administration. Capsules may have possible value in emergencies as a substitute for intravenous administration.

In our studies it was possible to investigate duration of toxic effects of cardiac glycosides in a small number of animals. Cats that recovered from the oral treatment were killed by intravenous injections of digitalis tincture. Four of the cats listed in Table I were permitted to recover for long periods of time, ranging from nineteen to forty-one days, before the intravenous fatal doses were determined. They required an average of 60 mg./Kg. of digitalis compared with 62 mg./Kg. for the controls. Those cats having a shorter interval of time between oral and intravenous testing required smaller intravenous lethal doses. This would seem to indicate that toxic doses of cardiac glycosides cause damage of a temporary rather than of a permanent nature.

SUMMARY

Four cardiac glycosides, digitoxin, digoxin, acetyl digoxin, and lanatoside C, were tested in immobilized unanesthetized cats. The drugs were administered orally in doses intended to be fatal, and survival time observed.

Vomiting did not occur, except in one animal, during the seven- to ten-hour observation periods on the animal boards. Loose stools in animals given high doses of lanatoside C indicated cathartic instead of vomiting action.

The drugs were given in solution form and in tablet form. Solutions of the drugs were more rapidly absorbed than the solid forms. Lanatoside C was incompletely and more slowly absorbed even in solution than the other glycosides. Capsules containing molecularly dispersed forms of cardiac glycosides in "Tween 80" would offer advantages over tablets for human therapeutic use according to the superior absorption rates in cats. The maintenance dose of digoxin in "Tween 80" in capsules would be estimated to be one-fifth of the present dose of 0.5 mg. for tablets, if absorption in humans is similar to that in cats.

Acetyl digoxin compares favorably with digitoxin in respect to absorption, both in solid form and in solution.

The relationship between lipoid solubility and rate of absorption from the intestinal tract is discussed.

Cardiac glycosides in toxic doses seem to cause damage to the heart of a temporary rather than of a permanent nature.

REFERENCES

(1) Diefenbach, W. C., and Meneely, J. K., Jr., *Yale J. Biol. Med.*, **21**, 421(1949).
(2) Gold, H., Kwit, N. T., and Cattell, M., *J. Pharmacol. Exptl. Therap.*, **69**, 177(1940).
(3) Gold, H., *et al.*, *ibid.*, **98**, 337(1950).
(4) Batterman, R. C., and DeGraff, A. C., *Federation Proc.*, **4**, 112(1945).
(5) Gisvold, O., and Hopponen, R. E., in press.
(6) Gisvold, O., and Krishnamurty, G. G., in press.
(7) Höber, R., *et al.*, "Physical Chemistry of Cells and Tissues," The Blakiston Company, Philadelphia, 1945.
(8) Chen, K. K., *Ann. Rev. Physiol.*, **7**, 677(1945).
(9) Chen, K. K., private communication.
(10) Stoll, A., "The Cardiac Glycosides," The Pharmaceutical Press, London, 1937.
(11) Clark, A. J., "The Mode of Actions of Drugs on Cells," The Williams & Wilkins Co., Baltimore, 1933, p. 84.
(12) Ipsen, J., "Contribution to the Theory of Biological Standardization," Arnold Busck, Copenhagen, 1941.
(13) Sciarini, L. J., and Salter, W. T., *J. Pharmacol. Exptl. Therap.*, **101**, 167(1950).

Notes

Cultivation Studies of the Solanaceous Drugs. V. A Note on a Method for Increasing the Growth of *Hyoscyamus niger**,†

By WILLIAM LUOPA, Jr.,‡ and WILLIS R. BREWER§

OVER a period of several years' study of the drug plant *Hyoscyamus niger* (*Solanaceae*), commonly known as black henbane, observations by the authors have frequently pointed out that the annual variety, as well as the second year's growth of the biennial variety of this plant, produce a slender, spindly flower stalk, the weight of which exceeds its supporting strength (1–4). As the stems approach heights of 35 cm. or greater, they may tend to fall against neighboring plants in the plot or to fall to the soil surface. In this condition, the plants are not provided an optimum environment and may be expected to fall short of their maximum stem growth and leaf production.

In light of the tremendous increase in plant size and leaf production of the common garden tomato, also a member of the *Solanaceae* family, when its stem is given support by an adjacent upright stake, an experiment was begun in February, 1950, to determine the value of such support in improving the productivity of black henbane.

EXPERIMENTAL

Authenticated seed samples of *H. niger* (annual variety), obtained previously from the United States Department of Agriculture, Bureau of Plant Industry, Beltsville, Md., and from the University of Washington College of Pharmacy, Seattle, Wash., were used as the source of plant material. Mature plants exhibited the typical purplish-veined zygomorphic corolla described in the monograph of the United States Pharmacopeia, in addition to the official macroscopic and microscopic characteristics.

Seeds were sown into flats in the greenhouse on February 20, 1950, and the seedlings transferred to individual wooden plant bands on April 20. One week later, 60 of the plants were set in two experimental plots of 30 each outside the greenhouse. The plants were sustained by surface watering procedures and protected from beetle and aphis invasions by the use of an all-purpose dust and a nicotine sulfate spray. During the first week in June, 15 of the plants in each plot were tied with cotton twine to an adjacent, upright, supporting, wooden stake of 1.5 meters in length. At this time the plants were approximately of equal height—about 30 cm. During stem elongation additional ties were added to provide adequate support for the plants.

During the last week in July, when all plants were in flower, each was cut at its base immediately above the soil surface, measured for stem length, and dried on tables in a shaded area having an average temperature near 90° and an average relative humidity near 45%. When the leaves were dried sufficiently to crumble with slight digital pressure, they were stripped from each stem and a pooled sample was made of the leaves from the 15 stake-supported plants in each plot and another of the leaves from the 15 control plants in each plot. These four pooled samples were weighed, their average weight per plant calculated, and then ground to a moderately coarse powder for assay of total alkaloid content according to the official method of the U. S. P. XIII.

RESULTS

Table I shows the stem measurements of the stake-supported and of the control plants from each of the two experimental plots.

The data of Table I may be said to indicate a significant difference between the average stem

TABLE I.—BLACK HENBANE STEM HEIGHTS FROM STAKE-SUPPORTED AND CONTROL PLANTS

Supported			Controls	
Plot A, Cm.	Plot B, Cm.		Plot A, Cm.	Plot B, Cm.
77	85		31	39
69	81		29	45
78	87		43	40
81	88		41	43
84	98		41	42
75	87		36	38
72	93		32	47
68	86		37	42
77	89		43	45
79	81		40	41
87	84		34	44
78	82		38	46
84	90		37	43
83	93		31	44
78	81		42	46
—	—		—	—
78	87	Av.	37	43
82.5		Grand Av.	40.0	

* Received August 31, 1951, from the University of Arizona, College of Pharmacy, Tucson.
Presented to the Scientific Section, A. Ph. A., Buffalo meeting, August, 1951.
† An experiment carried out within the research facilities of the Pharmacognosy Department, University of Arizona.
‡ Student in the College of Pharmacy, University of Arizona.
§ Associate Professor of Pharmacognosy, University of Arizona.

heights of the supported and the control plants. The grand average of heights of the supported plants represents a growth increase of 106% over the grand average height of the control plants.

Table II shows the results of weighing the four pooled samples of dried leaves in determining the effect of the stem-supporting treatment on leaf production. The data of Table II indicate that there is a significant difference between the average dry weight leaf yield of the treated and of the control plants.

TABLE II.—COMPARISON OF ANNUAL HYOSCYAMUS LEAF PRODUCTION (DRY-WEIGHT) OF STAKE-SUPPORTED AND CONTROL PLANTS

Supported Plot A, Gm.	Supported Plot B, Gm.		Controls Plot A, Gm.	Controls Plot B, Gm.
180.0	208.5	Pooled samples of 15 plants	63.0	76.5
12.0	13.9	Av. per plant	4.2	5.1
12.95		Grand av. per plant	4.65	

The grand average leaf production by stake-supported plants represents an increase over that of the control plants of 178%, which is not altogether unexpected considering comparable results with stake-supported tomato plants.

There was no significant difference between the alkaloidal assays of the leaves of the treated and the control plants. The general average of all leaf assays of both groups of plants was 0.07+% which is indicative of good quality leaf inasmuch as the current official requirement of the N. F. IX for hyoscyamus is only 0.04% total alkaloids.

SUMMARY

As a result of the described experimental treatment of *Hyoscyamus niger* (annual variety) involving the supporting of stems during their growing period, the following conclusion based on observation data is made evident:

Stake-supporting the stems of black henbane during their period of growth may be expected to increase the stem length and leaf production significantly but may not be expected to alter the alkaloidal yield.

REFERENCES

(1) Brewer, W. R., and Hiner, L. D., THIS JOURNAL, **38**, 541(1949).
(2) Brewer, W. R., and Hiner, L. D., *ibid.*, **39**, 586(1950).
(3) Brewer, W. R., and Hiner, L. D., *ibid.*, **39**, 638(1950).
(4) Brewer, W. R., and Hiner, L. D., *ibid.*, **39**, 639(1950).

A Note on a Modification of a Method for Evaluating Salicyl-Type Analgetics*,†

By DONALD C. BRODIE, E. LEONG WAY, and GLENN E. SMITH, Jr.

WHEREAS many pharmacologic classes of agents can be studied by methods that permit reasonably well-defined end points, the study of "analgetic" potency in pain-relieving drugs is particularly difficult because reliable methods for screening and study are not available. Discussions of available methods for the study of analgetic potency have appeared elsewhere (1, 2), but in general a suitable method should meet the following qualifications: (*a*) it should measure the sensation of pain or a sensation associated with pain; (*b*) it should produce a stimulus that will provoke a well-defined and uniform response; (*c*) it should permit duplication in the degree of response in keeping with that expected in biological experimentation; (*d*) it should be sensitive; (*e*) it should be relatively simple in design and in application; (*f*) it should be applicable to small animals for reasons of economy and convenience; and (*g*) it should permit qualitative and, if desired, quantitative evaluation of the results. This paper is a report of a method designed to permit screening of a series of compounds related chemically to salicylamide. The latter compound, known for more than 100 years, has only in recent times been recognized as a useful analgetic (3–6). Although no claim is made that this method meets all the qualifications outlined above, it was found suitable for a preliminary evaluation of a series of salicyl-type compounds.

Analgetic potency was determined by measuring the grams of force necessary to elicit a cry response from rats when a sharp stimulus was applied to the area of the tail 1–2 cm. from the base. The instrument used (Fig. 1) was a modification of one originally described by Eagle and Carlson (7). A standard aluminum welding rod, $13^1/_4 \times {}^1/_4$ in., tapered at one end to a sharp point was mounted in the following assembly: the outer housing was a piece of brass tubing, $7^1/_4 \times {}^1/_4$ in. (inside diameter), the ends of which contained a brass bushing which permitted the encasement of two serially-placed coiled springs[1] and through which the aluminum rod was placed.

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[1] Made by Instrument Specialties Company, Little Falls, N. J.; beryllium-copper wire, 0.0201-in. diameter; stiffness, 2.9 oz. per in.; spring No. 137-94; each segment, $3^1/_2$ in. long.

Figure 1

A small washer was placed in the bottom of the tube and pinned to the rod. Pressure exerted at the point of contact with the rat's tail, compressed the spring, the degree of compression being read in grams from a calibrated scale on the upper end of the rod. The scale had been calibrated in 10-Gm. increments and permitted readings that ranged from 10 to 170 Gm. A zero reading was impossible because of the combined weight of the rod and the washer. The above assembly was then placed in a large cork suitably drilled and mounted in a microscope so that the mount rested in the vertical tube from which the eyepiece, the revolving nosepiece, the stage, and the mirror had been removed. This arrangement made it possible to control and to duplicate the intensity of pressure resulting from a single stimulus by raising and lowering the assembly. Rats were placed in individual wooden cages with adjustable end gates which permitted the extension of the rat's tail. The response obtained with young Wistar rats, 40–60 Gm., was of sufficient uniformity to permit both qualitative and quantitative evaluation of the data.

Each animal was placed in a cage ten or fifteen minutes before testing to permit adjustment to the surroundings. Control data on each rat were obtained by recording the weight necessary to elicit a pain response as evidenced by either a cry or some act of obvious discomfort. Three consecutive readings agreeing within 5 Gm. of the average of the three readings was chosen as the normal threshold for an animal. Control values ranged from 10 to 30 Gm. and animals whose response did not fall within the above range were discarded. Similarly those rats that were extremely sensitive to even a touch stimulus were regarded as unsuitable for use. After determining the control data for a given animal, the drug was administered. At fifteen-, thirty-, and sixty-minute intervals, the pain-response threshold was again determined with each animal in which an attempt was made to get a response in four out of five successively applied stimuli of the same intensity. For rough screening purposes, the following system was chosen: 0–14 Gm., no effect "O"; 15–29 Gm., slight effect, "S"; 30–49 Gm., moderate effect, "M"; 50 Gm. and above, pronounced effect, "P." The selection of the 50-Gm. limit for maximal analgetic potency was an arbitrary choice in order to minimize the likelihood of trauma. Furthermore, it was difficult to obtain duplicate values when the stimulus-provoked response was greater than 50 Gm. When morphine, codeine, demerol, and salicylamide were tested using the above method, the response to the stimulus was almost invariably a cry, whereas rats receiving sodium pentobarbital usually reacted with an escape-like response when the degree of depression was one of lethargy. Salicylamide in intraperitoneal doses of 100 mg./Kg. and 200 mg./Kg. produced values of "S" and "P," respectively.

For a more quantitative evaluation, an arbitrary 25-Gm. increase in pressure was chosen. Dose-response relationships were determined by selecting a series of doses for a given compound, and obtaining an all or none effect in a series of animals using the above end point. These data will be reported at a later date.

REFERENCES

(1) Goetze, F. R., Burrill, D. Y., and Ivy, A. C., *Quart. Bull. Northwestern Univ. Med. School*, **17**, 280(1943).
(2) Chen, K. K., THIS JOURNAL, **38**, 51(1949).
(3) Ichniowski, C. T., and Hueper, W. C., *ibid.*, **35**, 225(1946).
(4) Hart, E. R., *J. Pharmacol. Exptl. Therap.*, **89**, 205(1947).
(5) Litter, M., Moreno, A. R., and Donin, I., *ibid.*, **101**, 119(1951).
(6) Seeberg, V. P., Hansen, D., and Whitney, W., *ibid.*, **101**, 275(1951).
(7) Eagle, E., and Carlson, A. J *ibid.*, **99**, 450(1950).

Adsorption Characteristics of Aureomycin and Terramycin on Aluminum Hydroxide Gel and on a Bismuth Subsalicylate Preparation*

By H. E. PAUL and C. M. HARRINGTON

A bismuth subsalicylate preparation and aluminum hydroxide gels were compared with respect to their adsorption of antibiotics. Adsorption did not occur on bismuth subsalicylate; however, significant adsorption occurred with the aluminum hydroxide gels.

DiGangi and Rogers (1) have shown, and Greenspan, *et al.* (2), have confirmed, that aluminum hydroxide gel removes nearly all the activity of aureomycin by adsorption on the aluminum hydroxide. While Greenspan, *et al.* (2), have shown that the adsorbed antibiotic is not inactivated or destroyed *in vitro*, Waisbren and Hueckel (3), Seed and Wilson (4), and others have demonstrated that aureomycin is not released *in vivo*, since greatly reduced blood levels were observed when aluminum hydroxide was administered with the antibiotic.

Exploratory studies in our laboratory on the adsorption of aureomycin on reconstituted U. S. P. dried aluminum hydroxide gel, aluminum hydroxide gel, and a preparation of dihydroxy aluminum amino acetate N. N. R. indicated losses of 35, 90, and 50%, respectively.

Since at least one article (5) has appeared advocating the use of a bismuth subsalicylate preparation in conjunction with aureomycin to prevent nausea, it seemed advisable to study the possible adsorption of antibiotics by bismuth subsalicylate.

In our studies we have used two antibiotics, aureomycin HCl (Lederle) and terramycin HCl (Pfizer). The adsorbing properties of typical aluminum hydroxide gel and a bismuth subsalicylate preparation[1] have been compared using a modification of the method of DiGangi and Rogers (1).

PROCEDURE

Antibiotics were made up in 1 mg./ml. solutions in distilled water. Aluminum hydroxide gel was added to each of the antibiotic solutions in the proportion of 1:10. The bismuth subsalicylate preparation was added to each in the proportions of 1:10 and 1:1. Similar quantities of water were added to the control solutions. All solutions were shaken gently for one hour at room temperature. Aliquots were removed from the 1:10 aluminum hydroxide-antibiotic solutions and from the 1:10 bismuth subsalicylate-antibiotic solutions and centrifuged for ten minutes. Controls, uncentrifuged mixtures, and the supernatant from the centrifuged mixtures were diluted suitably, and tested for antibacterial activity by a modification of the F. D. A. cup-plate test. Zone edges are not sharply delineated, but by using a standard on each plate, activity may be readily estimated. The cup plate method was chosen because it permits comparison of activity of cloudy solutions. The results of this study are shown in Table I.

TABLE I

Preparations Tested	Aureomycin, % Loss	Terramycin, % Loss
Aluminum hydroxide gel, 1:10, not centrifuged	50	25
Aluminum hydroxide gel, 1:10, centrifuged	Over 90	50
Bis. subsal. preparation, 1:10, not centrifuged	0	0
Bis. subsal. preparation, 1:10, centrifuged	0	0
Bis. subsal. preparation, 1:1, not centrifuged	0	0
Bis. subsal. preparation, centrifuged	0	..

It is to be noted that the uncentrifuged preparations of the antibiotics show less activity loss, indicating some elution during the cup-plate test.

Since the bismuth subsalicylate preparation has a lower *p*H (3.3 to 3.9) than the aluminum hydroxide gel (6.4), it was advisable to conduct a study in which the bismuth subsalicylate-antibiotic solutions were adjusted to the same final *p*H as the aluminum hydroxide gel-antibiotic solutions. Under these conditions there was still no adsorption of the antibiotics by the bismuth subsalicylate preparation.

CONCLUSION

The bismuth subsalicylate preparation studied does not inactivate either aureomycin HCl or terramycin HCl. Both aureomycin HCl and terramycin HCl are adsorbed on aluminum hydroxide gel in amounts ranging from 25 to 90%.

REFERENCES

(1) DiGangi, F. E., and Rogers, C. H., THIS JOURNAL, **38**, 646(1949).
(2) Greenspan, R., MacLean, H., Milzer, A., and Necheles, H., *Am. J. Digestive Diseases*, **18**, 35(1951).
(3) Waisbren, B. A., and Hueckel, J. S., *Proc. Soc. Exptl. Biol. Med.*, **73**, 73(1950).
(4) Seed, J. C., and Wilson, C. E., *Bull. Johns Hopkins Hosp.*, **86**, 415(1950).
(5) Wright, L. T., Strox, S., Marks, J. A., *Ann. West. Med. Surg.*, **4**, 717(1950).

* Received August 22, 1951, from the Norwich Pharmacal Company, Norwich, N. Y.
[1] Pepto-Bismol (trade mark of the Norwich Pharmacal Co., Norwich, N. Y.)

Book Reviews

Antibiotic Therapy. By HENRY WELCH and CHARLES N. LEWIS. The Arundel Press, Inc., Washington, D. C., 1951. xiv + 562 pp. Illustrated. 15 x 22 cm. Price $10.

Here is a book which should be of considerable interest to practicing pharmacists and to pharmaceutical chemists. The first half of the book, particularly, includes information on tyrothricin, penicillin, streptomycin, dihydrostreptomycin, bacitracin, aureomycin, chloramphenicol, terramycin, polymyxin, neomycin, mycomycin, viomycin, and subtilin, of special interest and use to practicing pharmacists. The first chapter of the book deals with the isolation and the development of the antibiotics and includes definitions of terms. Chapters 2 to 9, inclusive, are designed to summarize essential information on the antimicrobial spectrum, the pharmacology, and the dosage forms available for each antibiotic discussed. Each chapter is preceded by a photograph and a short biographical sketch of the discoverer of each antibiotic. In the third part of the book will be found a discussion of the treatment of specific diseases, in which the experience and results of the use of the several antibiotics are summarized.

The book is documented with significant references, and is well indexed to facilitate the location of specific information quickly. Dr. Welch, who is Director, and Dr. Lewis, who is Medical Officer in the Division of Antibiotics of the Food and Drug Administration, have brought together in their book an enormous amount of information concerning the antibiotics in common use. Through the design of the book and the style of presentation it can be used advantageously by practicing pharmacists as an authoritative source of information upon which to base answers to the many questions asked them by physicians relating to the present status of individual antibiotics. The information on dosage forms alone would warrant acquisition of the book, but in addition to this, the reader is supplied with answers to questions on indications and contraindications for use in the treatment of many infections, desirable routes of administration, the danger of the development of resistant strains of infective organisms, determination and maintenance of bactericidal blood levels, incidence and nature of allergic and other side reactions, and many other phases of the use of antibiotics with which the well-informed pharmacist should be familiar.

Pharmaceutical chemists may be disappointed that the chemistry of the antibiotics is not discussed. It should be borne in mind that this phase of the subject is too extensive in scope to be included in a book limited to antibiotic therapy.

The book can be unqualifiedly recommended as a guide and source of information to all who require more than a superficial knowledge of the many problems involved in the rational use of antibiotics in the treatment of disease.

Federal Food, Drug, and Cosmetic Act Judicial and Administrative Record, 1949-1950. By VINCENT A. KLEINFELD and CHARLES WESLEY DUNN. Commerce Clearing House, Inc., Chicago, 1951. 543 pp. 17.5 x 25.3 cm. Price $10.25.

This is the second of a series, the first of which was published early in 1951. [See THIS JOURNAL, **40**, 304(1951).] Like the first book in the series, this new one is logically arranged and clearly written and will be useful to anyone whose business or profession is regulated by the Act. One part of the book contains all opinions and decisions not otherwise generally available, except in Food and Drug Administration Notices of Judgment and in the loose-leaf service of the Commerce Clearing House Food, Drug, Cosmetic Law Reports. This material consists of summaries, each of which includes specific references to the various subsections of the Act involved. It also includes significant opinions under the 1906 Act not included in the first book covering the period 1907–1949.

The second part of the book includes "statements of general policy or interpretation." This section is especially valuable in indicating the attitude of the Food and Drug Administration, and reasoning by analogy it makes it possible for the reader to predict what the attitude of the F. D. A. will be on comparable problems. In the third section certain definitions and standards for foods promulgated by the Federal Security Administrator are reproduced in full. The value of the book both to professional and lay readers is enhanced by a table of references from law to interpretations, a cumulative table of cases, and a detailed index. The authors deserve special credit for planning the design of the book and writing the several summaries in clear and unambiguous style, and the publishers are to be complimented upon the excellence of the typography and the printing and binding.

Metabolic Methods (Clinical Procedures in the Study of Metabolic Functions). By C. FRANK CONSOLAZIO, ROBERT E. JOHNSON, and EVELYN MAREK. C. V. Mosby Company, St. Louis, 1951. 471 pp. Illustrated. 18 x 25.5 cm. Price $6.75.

In this book methods are presented in detail which have proved useful to the authors in research on various aspects of mammalian metabolism. The collection and storage of specimens, instrumentation methods, biochemical procedures, microbiological procedures, and physiological measurements are discussed in the first six sections of the book. Studies in the field, techniques for metabolic wards, and clinical laboratory procedures are discussed in the next three sections. In Section 10 references to standard works on statistical methods are listed, and in Section 11 considerable miscellaneous data of use to research workers in mammalian metabolism are included in tabular form.

Phase Microscopy—Principles and Applications. By ALVA H. BENNETT, HELEN JUPNIK, HAROLD OSTERBERG, and OSCAR W. RICHARDS. John Wiley & Sons, Inc., New York, 1951. xiii + 320 pp. Illustrated. 15.5 x 23.5 cm. Price $7.50.

This book is the first publication to present a working knowledge of the phase method, a technique which makes possible the microscopy of materials, inherently low in contrast. The four authors of this book, all of whom are with the Stamford Research Laboratory of the American Optical Company, have been actively engaged in original investigations in this field since Professor F. Zernike, of the University of Groningen, announced the microscopic application of the phase contrast method in 1935.

The authors have made significant contributions to the theory, instrumentation, and application of phase microscopy, and separate chapters in this book have been written by the one who has specialized in the particular division covered. The results of the authors' investigations are presented in such a manner that they can be applied to a wide variety of problems confronting microscopists.

Chapter I, by A. H. Bennett, presents the general aspects and correlations of the subject and gives the basic optical principles involved in the attainment of useful results with this relatively new technique. An elementary theory of phase microscopy is presented by H. Osterberg in what is said to be a simplified form in Chapter II, with a more detailed mathematical treatment of the diffraction theory of image formation in the phase microscope when used in conjunction with the commonly used Köhler method of illumination given in an appended Chapter VII.

The instrumentation necessary for the application of phase microscopy is described by Helen Jupnik in Chapter III. This includes the necessary alignment and adjustment of the microscope so that the most satisfactory and meaningful images can be obtained. Equipment supplied by different manufacturers is compared. Chapters IV, V, and VI, by O. W. Richards, deal comprehensively with the applications of phase microscopy in the fields of biology, medicine, and industry. Typical examples are given with materials for which the older microscopic methods are insufficient. An extensive bibliography, and author and subject indexes are appended.

This book should become indispensable to those who are interested in the development and the applications of this technical field of microscopy.

Physical Biochemistry. 2nd ed. By HENRY B. BULL. John Wiley & Sons, Inc., New York, 1951. ix + 355 pp. 15.5 x 23.7 cm. Price $5.75.

This book is intended to bring to the student of the biological sciences an understanding of physical chemistry, especially as it is applied to living systems. The author believes that a knowledge of physical chemistry is of greater assistance in the development of a background that will enable graduate students to integrate the scattered fragments of their knowledge of the basic sciences than any other study. This will not be disputed by any physical chemist.

The first edition of this book was reviewed in THIS JOURNAL, **33**, 160(1944). This second edition continues the policy of including only that material which has been useful and significant in solving professional problems. The interrelationship of the physical and biological sciences is stressed again and again throughout the text. The important modern methods and instruments of research, including electrophoresis, light scatter, and the use of isotopes as tracer elements are discussed. The applications of dielectrics, electrokinetics, viscosity, diffusion, and membranes are fully developed. Much of the material in the first edition has been rewritten and recent information has been incorporated in this edition.

References are given at the bottom of the page on which they are mentioned. Problems and questions are given at the end of each chapter, and, when mathematical calculations are involved, the answers are included.

Dr. Bull is a recognized authority in the field he has treated in this text, especially in the biochemistry of the lipids. He brings to the attention of the reader the impressive, the pioneering, and the splendid work of others in the field. His excellent treatise will be appreciated by students in the medical and biological sciences.

Approved Laboratory Technic. 5th ed. By JOHN A. KOLMER, EARLE H. SPAULDING, and HOWARD W. ROBINSON. Appleton-Century-Crofts, Inc., New York, 1951. xii + 1180 pp. Illustrated. 17.5 x 25.3 cm. Price $12.

The quotation, "No laboratory examination can be better than the thoroughness and skill with which it is conducted" sets the tempo for this excellent book on clinical laboratory tests and procedures. The three co-authors have enlisted the collaboration of 18 specialists in the revision of this comprehensive text. Special contributions were made by Chris J. D. Zarafonetis in "Methods for the Examination of the Blood"; by Morton Klein in "Diagnostic Virologic Methods"; by Edwin S. Gault and Anthony J. Lamberti in "Methods for Parasitologic Examinations"; and by Israel Davidsohn in "Hemagglutination and Blood Grouping."

The first part of the book is devoted to a thorough review of laboratory techniques, especially as regards the care and use of the microscope, the care of laboratory animals, and the symptoms of the diseases of different laboratory animals. Methods for the prevention and treatment of laboratory accidents are presented in a separate chapter. This serves to emphasize the importance of the information, and the reports of accidental infections among experienced laboratory technicians indicates the need for even greater safety measures in bacteriologic and pathologic laboratories.

The methods given in this text are described in great detail, and the operator is requested to follow the printed procedure exactly. Proper stress is laid upon the accurate preparation of reagents, for the results of a test can be negated by inaccurate reagents as well as by poor laboratory technique. Interlaboratory testing surveys have produced data that have been used to develop procedures that enable better agreement in results obtained with tests such as the serologic tests for syphilis.

Methods for the collection of specimens for laboratory examinations are described in detail. Laboratories are advised and urged to refuse to examine

improperly collected and preserved specimens when these might yield erroneous and misleading results. All the procedures throughout the text are abundantly illustrated with excellent cuts demonstrating handling technique and showing the equipment used.

Complete references are given at each point of mention in the text. New material in this revision includes a rapid system for routine urine analysis, the Papanicolaou cytodiagnosis of cancer, determination of spinal fluid protein, pregnancy test with frogs, Isenberg's method for staining spermatozoa, Alving and Miller's inulin clearance and the thymol turbidity tests for liver function, tests for bacterial susceptibility to antibiotics and assay for antibiotics in body fluids, Rh factor tests, slide flocculation and micro- and macroflocculation tests for syphilis, flame photometry, and skin tests for histoplasmosis and drug allergies.

This book is a must for libraries available to clinical laboratory workers, and its thoroughness makes it especially suitable as a text for the instruction of students in this field.

Pharmacognosy. By ROBERTSON PRATT and HEBER W. YOUNGKEN, Jr. J. B. Lippincott Company, Philadelphia, 1951. xi + 644 pp. Illustrated. 15.5 x 23.5 cm. Price $8.50.

This book presents an entirely new approach to the subject of pharmacognosy. The authors consider pharmacognosy to be essentially a biochemic science that is based on botany, chemistry, biochemistry, plant physiology, and related sciences. Following this concept, the emphasis throughout the book is on chemical, physical, and physiological principles and their integration and application. The authors have succeeded admirably in presenting information so that the student may acquire an understanding of the factors involved in the production and use of plant and animal drugs. The book is divided into six parts. In the first part the general aspects of pharmacognosy, definitions, and sources of drug materials are explained. Part 2 presents an excellent introduction to cellular chemistry, and in Part 3 the utilization and classification of drugs are presented in an interesting style. Part 4 includes an adequate discussion of products of biosyntheses, certain of their derivatives, and their physiologic and pharmaceutical applications. In Part 5 the production, preservation, and evaluation of crude drugs are outlined under the heading "Practices and Procedures." Part 6, consisting of over more than 50 pages, treats the subject of pest and weed control and other problems in public health.

The book is both interesting and inspiring. It recognizes that the classical ideas of pharmacognosy no longer possess the significance they did thirty or more years ago. The fact is recognized, and emphasized throughout the book, that plant products will probably continue to play an important role as a source of drugs, but the background information relating to these drugs from plants, is far more realistic than the conventional type of pharmacognosy that has heretofore been required in schools of pharmacy. It is hoped that this new approach to an important pharmaceutical subject will inspire teachers of pharmacognosy to emulate the design outlined by the authors for teaching pharmacognosy with a modern outlook, and a consideration of the needs of the present generation of pharmacy students.

Statistical Methods for Chemists. By W. J. YOUDEN. John Wiley & Sons, Inc., New York, 1951. x + 126 pp. 15.5 x 23.5 cm. Price $3.

This book is written for chemists who make measurements and interpret experiments. It is unique in that no space is given to the presentation of statistical theory and proofs, which can be found in the listed books on statistics. These and other omissions from the text reflect the author's opinion that many of the ordinary situations confronting the scientist require knowledge of only a few statistical techniques. His stated purpose is to make available to the scientists, many of whom are inarticulate in the explanation of their results, the modern statistical system of units for expressing scientific conclusions. This is done with particular effectiveness through the use of actual data from experimental observations at the National Bureau of Standards.

Dr. Youden states that by means of statistical techniques the requisite latitude in making an estimate can be specified in terms of the quantity of data available. Statistical verdicts may be regarded as mathematically objective judgments based upon the numerical relationships among measurements. Arguments are given for and against the value of statistical considerations of data with the affirmative winning handily. It is stressed that the detection and elimination of inaccuracy are experimental tasks, not statistical ones. It follows, therefore, that false data caused by manipulative errors should be eliminated before the degree of precision is determined statistically.

The text material is presented clearly and the examples are well chosen. The headings of the 10 chapters indicate the scope of the text: (1) Precision and Accuracy; (2) The Measurement of Precision; (3) The Comparison of Averages; (4) The Resolution of Errors; (5) Statistics of the Straight Line; (6) The Analysis of Variance; (7) Interaction between Factors; (8) Requirements for Data; (9) Arrangements for Improving Precision; and (10) Experiments with Several Factors.

An index and tables of critical values of t and F and a table of squares are included.

Biological Antagonism. By GUSTAV J. MARTIN. The Blakiston Company, Philadelphia, Pa., 1951. vii + 516 pp. 16 x 23.5 cm. Price $8.50.

In this book, Dr. Martin reviews biological antagonism as reflected in natural and synthetic displacing agents, covers antagonism as seen in the fields of amino acids, purines, pyrimidines, vitamins, hormones, minerals, and as it forms the basis of immunology, pharmacology, and chemotherapy. Finally, he offers the concept of biological relativity based upon his extensive study of these biological antagonisms.

"Biological antagonism is a new science, bringing within its scope elements of biochemistry, pharmacology, chemotherapy, immunology, etc. It is based upon a functional concept which is fundamental to every biological science. Life exists because of biological antagonisms. This is an extremely broad statement but reflects no more than the fact that biological systems are orderly and that orderliness could not be except for antagonisms," according to Dr. Martin.

Calendar of the Pharmaceutical Society of Great Britain, 1951-1952. The Pharmaceutical Society of Great Britain, London, 1951. 308 pp. 14.5 x 22.5 cm. Price 12*s.* 6*d.*

According to one British meaning of the term, a calendar is an official publication giving regulations and announcements. The *Calendar of the Pharmaceutical Society of Great Britain for 1951–1952* is packed with useful information about the Pharmaceutical Society of Great Britain. This Society was organized in 1841, and granted a charter of incorporation in 1843. According to the 1951–1952 *Calendar*, "The constitution established by the Charter and the duties and powers given to the Society have been varied and elaborated by a series of Acts of Parliament culminating in the Pharmacy and Medicines Act, 1941. These Acts established pharmacy in Great Britain as a self-governing profession controlling its own educational policy, the standard of its examinations, the maintenance of the register of its members, the inspection of their business premises and their disciplinary control, subject to such government supervision as would ensure the proper carrying out of those duties."

From the above quotation, it is apparent that the Pharmaceutical Society of Great Britain possesses infinitely greater authority than any comparable organization in the United States. Because of its legislative and law enforcement authority, membership in the Society is a requirement for the practice of pharmacy. The issuance of an annual calendar accordingly appears to be indicated in order to keep practicing pharmacists informed, not only on matters related to the administration of the Society, its activities, and its educational requirements, but, in addition, to furnish legal information with which pharmacists are required to be familiar.

About half of the *Calendar* (137 pages) is devoted to general information relative to the administration and activities of the Society. In the remainder of the book, legal information including statutes, regulations, and legal decisions, are spelled out in detail. *The Calendar of the Pharmaceutical Society of Great Britain* must serve as a very useful reference book for pharmacists and others in Great Britain who have anything to do with the distribution of drugs and poisons. The book should also be of considerable interest to pharmaceutical manufacturers and pharmacists in this country who may wish to acquire a knowledge of the structure of the Society and the laws of Great Britain regulating the practice of pharmacy.

The Enzymes—Chemistry and Mechanism of Action. Vol. II, Part 1. Edited by JAMES B. SUMNER and KARL MYRBÄCK. Academic Press, Inc., New York, 1951. xi + 790 pp. 16 x 24 cm. Price $14.80.

This book represents Part 1 of Volume II of a set of books planned to survey the field of enzymology. Volume I, Part 1 was reviewed earlier [THIS JOURNAL, **40**, 178(1951)], and a review of Volume I, Part 2 appeared later [THIS JOURNAL, **40**, 477(1951)]. As in the first volume, each chapter is written by an authoritative worker in the particular field covered. The subjects discussed in Part 1 of Volume II are: Theory of Oxidation-Reduction; Thermodynamics and Mechanism of the Phosphate Bond; Transphosphorylating Enzymes of Fermentation; Dephosphorylation of Adenosine Polyphosphates; Aldolase and Isomerase; Keto Acid Decarboxylases; Amino Acid Decarboxylases; Desulfinase; Codehydrogenases I and II and Apoenzymes; Succinic Dehydrogenase; Hydrogenase and Hydrogenlyase; Flavin-Containing Enzymes; The Iron-Containing Enzymes. A. Cytochromes; Copper Oxidases; Oxidation of Amino Acids; Oxidation of Amines; Lipoxidase or Unsaturated-Fat Oxidase; Enzymes in Luminescence; Oxidation of Organic Sulfur; Anaerobic Glycolysis, Respiration, and the Pasteur Effect; and Yeast and Mold Fermentations.

The appearance of this book will be welcomed by all who work in the field of enzyme chemistry, and those in other fields who need to acquire a knowledge of the chemistry and mechanism of action of the enzymes.

The Pectic Substances. By Z. I. KERTESZ. Interscience Publishers, Inc., New York, 1951. xvi + 628 pp. 16 x 23.8 cm. Price $13.50.

The author and the publisher of *The Pectic Substances* have performed a most useful service to research workers and technologists, in the fields of foods and drugs particularly, by making available this book covering all aspects of pectin and pectic substances. This is the first comprehensive monograph on pectic substances to be written. The book should be useful, not only to specialists in pectin chemistry, but to others interested in problems related in any manner to the pectic substances. The comprehensiveness of the book is indicated by the titles of the several divisions each of which consists of several chapters. In Part 1, 258 pages are devoted to the chemistry of pectic substances. In Part 2, the botany of these materials is adequately covered and in Part 3, biochemical aspects are discussed. The manufacture of pectic substances is described in Part 4, and in Part 5 some of the functions and applications of pectic substances and pectic enzymes are discussed.

The book is well documented with more than 2,000 references to the original literature and includes an author index and an excellent subject index. The printing and binding are of good quality. The book can be recommended highly to anyone having occasion to need information of any kind relating to the pectic substances.

PVP: Polyvinylpyrrolidone. Compiled and published by the General Aniline & Film Corporation, 230 Park Ave., New York, 1951. xxviii + 174 pp. Illustrated. 15.5 x 23.5 cm.

Among the several so-called "blood substitutes," one which has attracted considerable attention is polyvinylpyrrolidone, commonly referred to as PVP. This product was used extensively by German army physicians and surgeons as an emergency blood substitute during World War II. During the years since its introduction in 1941, many papers have been published relating to PVP, not only as a blood substitute, but as a therapeutic agent. This book consists of an annotated bibliography, in which 227 papers are abstracted. The book includes an author index, a numerical patent index, and the abstracts are arranged according to the particular application of PVP to which they refer.

Organic Chemistry. By J. P. WIBAUT. Elsevier Publishing Company, Houston, Texas, 1951. xiv + 660 pp. 18 x 26 cm. Price $9.

During the first three decades of this century, the English translation by A. J. Walker and O. E. Mott, of the *Textbook of Organic Chemistry*, by A. F. Holleman, was widely used in colleges in the United States. The new edition retains much of the original character of the work, and the subject continues to be treated both from an organic and from a physiochemical standpoint. The great advances in the science of organic chemistry, however, are reflected throughout the new edition. As might be expected, aliphatic, aromatic, and heterocyclic compounds are arranged and discussed in a logical manner so that by use of the book students may gain a clearer knowledge of the problems of organic chemistry and its historical development. Of special interest to pharmaceutical, medical, and biochemic readers will be the unusually adequate treatment of important groups of natural products such as carbohydrates, proteins, terpenes, carotenes, sterols, alkaloids, vitamins, and hormones. Information relating to many of the antibiotics of importance in medicine is also presented. The penicillins, for example, are described as derivatives of thiazole.

The general make-up of the book is excellent, and like its precursors, it is admirably suited for use as a textbook and reference by students in the medical sciences as well as those specializing in organic chemistry.

A Dictionary of Antibiosis. Compiled by LEONARD KAREL and ELIZABETH SPENCER ROACH. Columbia University Press, New York, 1951. 373 pp. 16 x 24.3 cm. Price $8.50.

This book provides a comprehensive reference for workers and readers in the medical and biological fields of antibiosis. Among the alphabetically arranged entries are listings of plants of all forms from which antibiotic substances have been derived, microscopic forms used as test organisms, and the many familiar and strange specific antibiotic substances. Where the information has been obtainable for the individual antibiotic substance, the source of the substance, its method of extraction, chemical and physical properties, its antibacterial spectrum *in vitro* and *in vivo*, structural formulas, and toxicological, pharmacological, and clinical data are given.

The term antibiotic is defined as a substance derivable from living organisms and capable of adversely modifying the vital functions of specific microorganisms. Under the name of each of the test organisms given is listed, wherever applicable, the species tested against it for antibiosis, and the named antibiotics that have been tested against it. Where very long lists of substances have been tested against a given microorganism and the tests are reported in one paper, only the bibliographic reference is given. Strain designations are not routinely included. Ethyl alcohol and quinine, mentioned as two of the older antibiotics, have been omitted.

The rapid growth of the field of antibiosis and the need for a book like this dictionary is indicated by the 316 pages of condensed information, in addition to the 55 pages of alphabetically arranged bibliography. Complete titles of papers are given in the bibliography. This is helpful in the search for additional information. The changes in this field occur so rapidly that even the dictionary is confusing at times. Under polymyxins we are told that polymyxin A was formerly designated as aerosporin. That also informs us that this is no longer so. But it does not inform us that a polymyxin B sulfate preparation is now designated by the trade name Aerosporin.

This dictionary will be a very useful and valuable addition to any scientific library, and every worker in the field of antiobiosis will want to have a copy of this book available.

Six-Membered Heterocyclic Nitrogen Compounds with Four Condensed Rings. By C. F. H. ALLEN. Interscience Publishers, Inc., New York, 1951. xiii + 345 pp. 16 x 23.8 cm. Price $10.

This is the second volume of a series of monographs presenting a comprehensive treatment of heterocyclic chemistry. The first volume, devoted to the heterocyclic derivatives of phosphorus, arsenic, antimony, bismuth, and silicon, was published in 1950 and previously reviewed [THIS JOURNAL, **39**, 482(1950)].

The group of heterocyclic compounds discussed in the second volume is of interest to the drug and dye industries and also to organic chemists because of its practical, theoretical, and historical importance. All of the important heterocyclic nitrogen analogs of the seven different ring systems established by fusion of four benzene rings are discussed in the following sequence: naphthacene, benz[a]anthracene, benzo[c]phenanthrene, chrysene, triphenylene, benzanthrenes, and pyrene. The similarities and significant differences shown by the various members of the system are emphasized.

The "a" system of nomenclature is used throughout the text with the addition of the *Ring Index* number and the *Chemical Abstracts* name, both of which serve admirably as an aid to the reader. The book is well documented and the literature through 1950 is covered. The value of the book is enhanced by an excellent index.

Outline of Fundamental Pharmacology. By DAVID FIELDING MARSH. Charles C Thomas, Publisher, Springfield, Ill., 1951. xxxi + 219 pp. 16 x 23.5 cm. Price $6.

This book has been designed to explain the aims and purposes of the pharmacologist. It can be especially useful to research workers in medicinal chemistry, pharmaceutical chemistry, and related fields in which a knowledge of the basic principles of pharmacology is useful in understanding the steps through which a product must progress before it can be subjected to clinical trial. Among the subjects discussed are dose-effect relationship, localization of site of action, absorption and distribution, fate of drugs, mechanism of action, and chemical constitution and biological action. The book is generously documented with general references and a limited number of specific references are cited at the end of each chapter. The book is written in a clear, easily understandable style, and should be of especial value to workers in borderline fields as well as to professional pharmacologists.

The Molds and Man. By CLYDE M. CHRISTENSEN. University of Minnesota Press, Minneapolis, 1951. viii + 244 pp. 14.5 x 22.5 cm. Price $4.

The author of this book, Clyde M. Christensen, Professor of Plant Physiology at the University of Minnesota, has succeeded in presenting a scientific subject in an interesting, informative, and yet entertaining style. He discusses the basic facts about fungi in a manner that the average intelligent person, without a scientific background, can read and understand. He explains, among many other things relating to fungi, the commercial growth of some species of molds for the production of drugs, organic acids, and enzymes used in the processing of various foods. The book could be used as a text for an introduction to the basic principles of mycology. It is definitely of pharmaceutical interest because of the drug products described which are obtained from molds. The usefulness of the book is greatly enhanced by an excellent index.

Biochemistry and Physiology of Protozoa. Vol. I. By ANDRÉ LWOFF. Academic Press, Inc., New York, 1951. x + 434 pp. 15.5 x 23.8 cm. Price $8.80.

This volume includes a series of reviews dealing with the nutrition, metabolism, and growth of the Phytoflagellates, the parasitic flagellates and amebae, the Trypanosomidae, and the Bodonidae, the biochemistry of ciliates, and also the biochemistry of Plasmodium and the influence of antimalarials.

The publication of a second volume dealing with the physiology and biochemistry of other protozoa is planned. The first volume should be of especial interest to students and research workers in the field of biochemistry and related subjects.

Hydrogenation of Fatty Oils. By H. I. WATERMAN. Elsevier Publishing Company, Houston, Texas, 1951. ix + 254 pp. 15.5 x 23.5 cm. Price $6.50.

This book is intended to serve as a guide to further investigations and at the same time to furnish information of a sufficiently fundamental nature so that the reader may become familiar with the hydrogenation of fatty oils in a relatively short time. The chemical operations required and the raw materials used in the production of hydrogenated oils and fats are adequately discussed, and the chemistry involved is clearly explained. The book is provided with a bibliography, an author index, and a subject index, which enhances its usefulness.

Henderson's Materia Medica. By J. K. W. FERGUSON and G. H. W. LUCAS. University of Toronto Press, Toronto, 1951. viii + 314 pp. 15.5 x 23.5 cm.

This book contains brief descriptions of nearly all the drugs official in the British Pharmacopoeia, the United States Pharmacopeia, the British Pharmaceutical Codex, the National Formulary, and New and Nonofficial Remedies. It also includes a limited number of drugs not listed in any of the books previously mentioned. It might, under some circumstances, serve as a useful reference where the source books upon which it is based are not available.

Prescription Writing and Medical Jurisprudence. 4th ed. By HAROLD N. WRIGHT. Burgess Publishing Co., Minneapolis, 1951. iii + 114 pp. 21 x 27.8 cm. Price $2.25.

In the fourth edition of this well-known book by Dr. Harold N. Wright, of the Department of Pharmacology of the University of Minnesota, titles for drugs have been brought into conformity with those of the current editions of the United States Pharmacopeia and the National Formulary. Dr. Wright has also emphasized, to a greater extent than in previous editions, therapeutic considerations as the basis upon which prescriptions are written. In the medical jurisprudence section of the book, new information is presented on narcotic drugs covered by the Harrison Narcotic Act, and state laws relating to barbiturate and hypnotic drug distribution. It also includes pertinent information on prescription writing, including prescription Latin.

Bacterial Physiology. Edited by C. H. WERKMAN and P. W. WILSON. Academic Press, Inc., New York, 1951. xiv + 707 pp. Illustrated. 16 x 23.8 cm. Price $8.50.

This book is designed to provide the reader with modern views, theories, and discussions relating to bacterial physiology. The chemistry and the physiology of microörganisms have made great advances during recent years, and this book should go far to stimulate further research in this expanding branch of bacteriology. The several chapters of the book are written by 19 contributors, each of whom is a recognized leader in his respective field of investigation. The major portion of *Bacterial Physiology* is designed to provide a background knowledge of this science, while the rest of the book presents a series of short contributions illustrating the significance of bacterial physiology in the broader fields of general biology. The book should prove to be of especial interest to research workers in the fields of biochemistry, botany, and zoology, who wish to bring themselves up to date on the fundamentals of bacterial physiology.

Ultraviolet Spectra of Aromatic Compounds. By ROBERT A. FRIEDEL and MILTON ORCHIN. John Wiley & Sons, Inc., New York, 1951. 20.8 x 26.8 cm. Price $10.

While the main part of this book consists of a catalog of approximately 600 spectra of polynuclear aromatic compounds, it also includes succinct, but clearly written chapters on spectrophotometric nomenclature and the absorption law for solutions instruments and experimental procedure, theoretical aspects of ultraviolet absorption spectra, and the use of ultraviolet spectra in qualitative and quantitative analyses. All ultraviolet spectra reproduced have been transformed into a consistent method of plotting. The spectra of the compounds selected have been on their general or specific interest, and include many that are known to occur in coal tar and shale oil. The book should be helpful to workers just beginning to acquire a knowledge of spectrophotometric methods as well as to the organic chemist interested in using ultraviolet absorption spectra for the identification and quantitative estimation of organic compounds.

The Lipids: Their Chemistry and Biochemistry. Vol. I. By HARRY J. DEUEL, Jr. Interscience Publishers, Inc., New York, 1951. xxiv + 982 pp. 16.5 x 23.8 cm. Price $18.50.

In this volume the author, who is dean of the Graduate School and Professor of Biochemistry at the University of Southern California, has attempted to correlate the available information on the various components included under the broad and general classification of lipids. In the first volume only a chemical approach to the subject is included. Volume II, to be published later, will concern itself exclusively with the biochemical and nutritional considerations involving the lipids. The scope of the book can best be realized by the titles of the 10 chapters which are as follows: general characteristics and classification of lipids; the chemistry of fatty acids and glycerol; the chemistry of neutral fats; waxes, higher alcohols including sterols, triterpenes, glyceryl ethers, colored fats, and hydrocarbons; chemistry of the phosphatides and cerebrosides; carotenoids and related compounds; distribution, properties, and chemistry of the vitamins A; distribution, properties, and chemistry of the provitamins D and vitamins D; distribution, properties, and chemistry of the vitamin E group; and distribution, properties, and chemistry of the vitamin K group.

The book is written in a simple straightforward manner understandable to readers who are not necessarily specialists in the individual fields covered. The book is well documented, provided with an author index of plant and animal sources of lipids, and an unusually complete subject index.

The book should be useful to anyone requiring knowledge of the chemistry of the many products classified as lipids. The printing and binding of the book are up to the usual standard of excellence of Interscience Publishers.

Essentials of Pharmacology. 2nd ed. By FRANCES K. OLDHAM, F. E. KELSEY, and E. M. K. GEILING. J. B. Lippincott Co., Philadelphia, 1951. xvi + 462 pp. 13 x 20 cm. Price $5.

This book presents quite briefly certain essential information concerning many drugs in current use.

In each chapter a group of drugs possessing similar activity is discussed. A list of official preparations and a selected bibliography at the end of each chapter increases the significance and usefulness of the book for reference purposes. The book includes a discussion of the role of radioactive isotopes in medicine and medical research, a most comprehensive section on adrenergic blocking agents, a new chapter devoted to ganglionic blocking agents and muscle relaxants, and an extensive revision of the section on antihistaminics. New drugs such as cortisone, ACTH, and the antibiotics, are also included.

Physiology and Pharmacology for Pharmaceutical Students. 3rd ed. By HAROLD HAYDEN BARBER. Williams and Wilkins Co., Baltimore, 1951. x + 622 pp. Illustrated. 15 x 22.3 cm. Price $6.50.

This book was written specifically for pharmaceutical students in Great Britain, for their use in preparing for the final examinations of the Pharmaceutical Society of Great Britain, and the examinations for degrees in pharmacy in English universities. The title of the book is, in a way, misleading, because the greater emphasis is on physiology instead of on pharmacology. A detailed description of laboratory exercises in histology, experimental physiology, and physiological chemistry is included at the end of each chapter. The book is clearly written and includes a liberal number of illustrations which serve to add materially to the clarity of the text. Dr. Barber's book appears to be admirably suited for the teaching of a basic course in physiology to pharmacy students who do not intend to become professional pharmacologists.

Science French Course. By C. W. PAGET MOFFATT. Revised by NOEL CORCORAN. Chemical Publishing Co., Inc., Brooklyn, N. Y., 1951. viii + 332 pp. 12 x 19 cm. Price $4.75.

This book is designed for students having no previous knowledge of the French language, to enable them to acquire a reading knowledge of scientific and technical literature in that language. The first one-third of the book is devoted to the elements of grammar which are limited to the bare essentials. If this section is mastered, however, it should enable the student to progress fairly rapidly through increasingly difficult graded reading exercises.

Reading exercises of increasing difficulty covering every branch of science, make up the major part of the remainder of the book. For the graduate student in pharmaceutical chemistry, for example, the reading exercises in chemistry appear to be too few in number to insure a sufficient reading knowledge of chemical French to enable him to progress to the reading of original articles in French pharmaceutical and chemical journals. A detailed alphabetical vocabulary is provided which adds to the value of the book.

Pharmaceutical Dispensing. 4th ed. By WILLIAM HUSA. Distributed by Husa Brothers, College and Dubuque Sts., Iowa City, Iowa, 1951. vii + 811 pp. Illustrated. 16 x 23.6 cm. Price $6.

This book has undergone three revisions since the first edition was published in 1935. The style adopted for the first edition has been maintained in each revision, but the new fourth edition, like the second and third editions, shows that the latest revision has been unusually thorough. Much obsolete material has been deleted and replaced with new information and new prescriptions. Among some of the new material discussed in the text are soluble ointment bases, methylcellulose jellies, the use of blenders in the preparation of emulsions, a new type of mortar and pestle, the microfilming of prescriptions, and the receiving of prescriptions by wire recorder attached to the telephone. The chapter on institutional pharmacy has been revised by including new information relative to the practice of pharmacy in the Army and the Navy. The chapter on incompatabilities, which has been excellent in previous editions, has been brought up to date by the addition of new sections on antihistaminics, quaternary ammonium compounds, and other new drugs. Dr. Husa's book should find ready acceptance by educators, students, and practicing pharmacists.

Textbook of Pharmacognosy. 2nd ed. By T. E. WALLIS. J. and A. Churchill Ltd., London, 1951. xi + 556 pp. Illustrated. 15 x 23.5 cm. Price 35*s*.

The first edition of this book was thoroughly reviewed [THIS JOURNAL, **25**, 321 (1946)] and the second edition adheres to the original style previously described. The text, however, has been thoroughly revised by the addition of new illustrations and the inclusion of additional figures. Short articles describing pollen, mechanical wood pulp, regenerated cellulose, alginate fibers, and shellac, have also been added. Otherwise this new edition can be described as a typical textbook of pharmacognosy.

Preparation, Properties, and Technology of Fluorine and Organic Fluoro Compounds. CHARLES SLESSER, Editor, and STUART R. SCHRAM, Associate Editor. McGraw-Hill Book Co., Inc., New York, 1951. xxiii + 868 pp. Illustrated. 16 x 23.7 cm. Price $10.50.

This volume is one of a series intended to record the research work done under the Manhattan Project and the Atomic Energy Commission. The main purpose of this volume is to present information recently developed in the fields of fluorine and fluoro compound technology. The large-scale manufacture of fluorine, methods of handling it, and the preparation of new fluoro-organo compounds are described.

BOOKS RECEIVED

Penicillin Decade: A Report of Sensitizations and Toxicities, 1941–1951. By LAWRENCE WELD SMITH and ANN DOLAN WALKER. Arundel Press, Inc., Washington, D. C., 1951. 122 pp. 14.5 x 22 cm. Price $2.50.

Substances Naturelles de Synthese. Vol. III. By LEON VELLUZ. Masson & Cie, Paris, 1951. 156 pp. 16 x 23.3 cm. Price 1500 fr.

Federal Food, Drug, and Cosmetic Act Judicial and Administrative Record, 1949–1950. By VINCENT A. KLEINFELD and CHARLES WESLEY DUNN. Commerce Clearing House, Inc., Chicago, 1951. 543 pp. 17.5 x 25.3 cm. Price $10.25.

Diseases in Old Age. By ROBERT T. MONROE. Harvard University Press, 1951. xi + 407 pp. 15 x 21.8 cm. Price $5.

Ultraviolet Spectra of Aromatic Compounds. By ROBERT A. FRIEDEL and MILTON ORCHIN. John Wiley & Sons, Inc., New York, 1951. 20.8 x 26.8 cm. Price $10.

Early Arabic Glass Weights and Stamps: A Supplement. By GEORGE C. MILES. The American Numismatic Society, New York, 1951. 60 pp. Illustrated. 15.6 x 22.9 cm. Price $2.50.

Experiments in Biochemistry. By MAX S. DUNN and WILLIAM DRELL. McGraw-Hill Book Company, New York, 1951. viii + 197 pp. and data sheets. 22 x 28.8 cm. Price $5.

Prescription Writing and Medical Jurisprudence. 4th ed. By HAROLD N. WRIGHT. Burgess Publishing Co., Minneapolis, 1951. iii + 114 pp. 21 x 27.8 cm. Price $2.25.

Laboratory Guide in Pharmacology for Pharmacy and Dental Students. Revised ed. By HARALD G. O. HOLCK. Burgess Publishing Co., Minneapolis, 1951. xiv + 129 pp. Illustrated. 21 x 27.5 cm. Price $2.75.

The Pectic Substances. By Z. I. KERTESZ. Interscience Publishers, Inc., New York, 1951. xvi + 628 pp. 16 x 23.8 cm. Price $13.50.

Hormones: A Survey of Their Properties and Uses. Published by direction of the Council of the Pharmaceutical Society of Great Britain. The Pharmaceutical Press, London, 1951. xii + 220 pp. Illustrated. 14.5 x 22.8 cm. Price 35*s*.

PVP: Polyvinylpyrrolidone. Compiled and published by the General Aniline & Film Corporation, 230 Park Ave., New York, 1951. xxviii + 174 pp. Illustrated. 15.5 x 23.5 cm.

Accepted Dental Remedies. 17th ed. Council on Dental Therapeutics, American Dental Association, Chicago, 1952. xvi + 211 pp. 16 x 20.5 cm. Price $1.50.

Something Can Be Done About Chronic Illness. By HERBERT YAHRAES. The National Commission on Chronic Illness and the Public Affairs Committee, New York, 1951. 32 pp. Illustrated. 13.7 x 21.3 cm. Price 25¢.

Formulary and Therapeutic Guide. Compiled by the Formulary Committee of the New York Hospital. Appleton-Century-Crofts, Inc., New York, 1951. xv + 355 pp. 12.5 x 19.5 cm.

Antibiotic Therapy. By HENRY WELCH and CHARLES N. LEWIS. The Arundel Press, Inc., Washington, D. C., 1951. xiv + 562 pp. Illustrated. 15 x 22 cm. Price $10.

The Molds and Man. By CLYDE M. CHRISTENSEN. University of Minnesota Press, Minneapolis, 1951. viii + 244 pp. 14.5 x 22.5 cm. Price $4.

Bacterial Physiology. Edited by C. H. WERKMAN and P. W. WILSON. Academic Press, Inc., New York, 1951. xiv + 707 pp. Illustrated. 16 x 23.8 cm. Price $8.50.

Untoward Reactions of Cortisone and ACTH. By VINCENT J. DERBES and THOMAS E. WEISS. Charles C Thomas, Publisher, Springfield, Ill., 1951. ix + 77 pp. 14.5 x 22.5 cm. Price $2.25.

Statistical Methods for Chemists. By W. J. YOUDEN. John Wiley & Sons, Inc., New York, 1951. x + 126 pp. 15.5 x 23.5 cm. Price $3.

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